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Marine Biological Laboratory

WOODS HOLL, MASS.

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BIOLOGICAL BULLETIN

ON THE NORTH AMERICAN MARINE TRICLADS.

DR. J. WILHELMI.

During the last fifty years ten marine species of triclads were found on the east coast of North America:

Procerodes wheatlandi Girard (according to Verrill = *Procerodes ulvæ* (Oe.), according to Curtis = *Gunda segmentata* Lang).

Procerodes frequens Leidy (according to Verrill = *Procerodes wheatlandi* Gir.).

Fovia (*Vortex*) *warreni* (Gir.).

Fovia (*Planaria*) *grisea* (Verrill).

Fovia affinis (Oe.) (var. *warreni* and var. *grisea*).

Fovia (*Planaria*) *littoralis* (Verrill).

Bdelloura (*Vortex*) *candida* (Gir.) (= *Bdelloura parasitica* Leidy = *Planaria limuli* v. Graff).

Bdelloura propinqua Wheeler.

Bdelloura rustica Leidy.

Syncoelidium pellucidum Wheeler.

The identifications for the most part of these are quite doubtful, because of the incomplete description; also the identifications of these species with European species, given by some authors, are untenable. I studied these North American species in the most important localities and in the following paper I shall give a short notice of my results. I am not concerned with the Bdellouridæ (genera *Bdelloura* and *Syncoelidium*), living on *Limulus*, because these have already been well described by Wheeler.[1] *Bdelloura rustica*, a free-living form, described by Leidy[2] cannot be identi-

[1] Wheeler, W. M., "*Syncoelidium pellucidum*, a New Marine Triclad," *Journ. Morph.*, Boston, 1894, pp. 167–194, Pl. 8.

[2] Leidy, T., "Helminthological Contributions," No. 3, *Proc. Acad. Nat. Sc.*, Vol. V., Philadelphia, 1850/51, pp. 242, 243, 289.

fied as such and doubtless it does not belong to the genus *Bdelloura*. Also I have published a paper[1] on the larvæ of planarians, named by Agassiz[2] *Planaria angulata* and the probable confusion of these with young Bdellouridæ. The following communication concerns only the free-living marine triclads.

Girard[2] described in 1850 a marine triclad, found near Manchester (Massachusetts Bay) under the name *Procerodes wheatlandi* n. gen. n. sp. The same animal was found by *Leidy* (9) in 1855 near Point Judith, R. I., and was described as *Procerodes frequens* n. sp. Neither species was investigated in relation to the genital apparatus and nevertheless Verrill (10), in 1873, identified them with the North European species *Gunda ulvæ* (Oe.).[3] Girard[3] called the first species by its old name *Procerodes wheatlandi* but the second and the third (European) species he classed with the Rhabdocoelidæ (!) under the name of *Neoplana* n. g. *frequens* and *N. ulvæ*. Curtis[1] supposed that Verrill's *Procerodes ulvæ* (*Procerodes wheatlandi* + *Procerodes frequens*) may be identical with the South European *Procerodes* (*Gunda*) *segmentata* (Lang).

Without mentioning the mistake of Girard, there are two questions to answer: (1) Is *Procerodes wheatlandi* (*Procerodes frequens*) identical with one of the European triclads *Procerodes ulvæ* or *Procerodes segmentata?* (2) Must the European genus *Gunda* O. Schm. (+ *Haga* O. Schm.) be classed with the American genus *Procerodes?*

In relation to the last question Bergendal[4] correctly mentioned that for the present the well-described genus *Gunda* must be preferred to the insufficiently described genus *Procerodes*. Böhmig[5]

[1] Wilhelmi, J., "Über *Planaria angulata* Müller," *Zool. Jahrb.*, Abth. Sytematik, 26. Bd., 1907, 10 pp., 1 Taf.

[2] Agassiz, A., "On the Young Stages of a Few Annelids," *Ann. Sc. N. H.*, New York, 8. Bd., 1866, pp. 306–309, Taf. 1, figs. 1 u. 2.

[3] Oersted, A. S., "Forsog til en ny Classification of Planarierne (Planarica Dugès) etc.," *Kroyers Naturh. Tidsskrift*, IV., 1843, p. 551.

[4] Bergendal, D., "Studier öfver Turbellarier. 2. Om Byggnaden af *Uteriporus* Bgdl. jämte andra bidrag till Tricladernas anatomi." *Fysiogr. Tällsk Lund Handl.* (2), Bd. 7, 1896. — Ueber drei Tricladen aus Punta Arenas und umliegender Gegend," *Zool. Anz.*, 22. Bd., 1899.

[5] Böhmig, L., "Turbellarienstudien: *Tricladida maricola*," *Zeitschrift f. wiss. Zool.*, 81. Bd., 1896.

referred to the same question: "Die von Girard gegebene Charakteristik des Genus *Procerodes* ist eine sehr oberflächliche, sie bezieht sich nur auf das Extérieur, während die Beschreibung und Abbildungen O. Schmidt's (*Gunda*) genügend kennzeichnende sind. . . . Mit Rücksicht auf die grosse Uebereinstimmung, welche sich hinsichtlich der Form zwischen *Procerodes* und der überwiegenden Mehrzahl der *Gunda*-Arten ergibt, mit Rücksicht weiterhin auf den Umstand, dass wenigstens eine sichere *Gunda*-Species an der Nordamerikanischen Küste beobachtet wurde ("but it was not"), acceptiere ich die *Girard'sche* Bezeichnung, obwohl der Copulations-apparat von *Pr. wheatlandi* total unbekannt ist und den Zweifeln, die Bergendal bezüglich der Identität von *Procerodes* und *Gunda* äussert, eine Berechtigung nicht abgesprochen werden kann. Mit Sicherheit lässt sich diese Frage nur durch die Untersuchung der Originalexemplare von *Pr. wheatlandi* entscheiden, . . ."

I studied the above mentioned North American [1] and European [2] species and found that following Verrill the genus *Gunda* O. Schm. because of the agreement of the genital apparatus of *Procerodes wheatlandi* with this of European *Gunda*-Species must be classed with *Procerodes*. But *Procerodes wheatlandi* (and *frequens*) is not identical with *Procerodes* (*Gunda*) *ulvæ* although they closely resemble one another. Also Curtis' [1] supposition given in a short notice, "The occurrence of *Gunda segmentata* in America," that *Procerodes wheatlandi* may be identical with the South European species *Gunda segmentata*, and not, as Verrill supposed, with *Procerodes* (*Gunda*) *ulvæ*, is a mistake. *Procerodes segmentata* [3] is quite free from pigment and therefore, setting aside the different forms of the head will be easily distinguished from the North American *Procerodes wheatlandi* and the North European *Procerodes* (*Gunda*) *ulvæ*.

Nearly contemporary with the description *Procerodes wheat-*

[1] Collected at Cuttihunk (Elizabeth Islands) and Newport, R. I.; through the courtesy of Professor Curtis I obtained also the original material of his *Gunda segmentata* from Sandwich, Mass., where I myself unsuccessfully sought for these species. I found it in Buzzards Bay, near Sandwich, summer 1907.

[2] I collected it at Travemünde and Copenhagen in September, 1906.

[3] I collected it during the last three years at Naples and many other localities of the Mediterranean Sea.

landi Girard (3) described a second new marine triclad from Boston Bay, *Vortex warreni* n. sp., for which later he established the new genus *Fovia* (7). Verrill (10) in 1873–74 described the same species as *Planaria grisea* n. sp. and later as *Fovia littoralis* n. sp. (11). The descriptions of these species and of the genus *Fovia* are insufficient. Already in 1857 Stimpson[1] classed the European *Planaria affinis* Oe. with the genus *Fovia* and Verrill (12) identified the preceding North American species with Stimpson's *Fovia affinis* (Oe.) as varieties *warreni* and *grisea*. Girard (3) meanwhile cites *Fovia warreni* as a marine triclad (with anus) and classed the identical *Fovia grisea* with his new rhabdocœlid (!) genus *Neoplana*. From the North European *Fovia affinis* I studied the only three existing individuals of the Museum of Bergen and showed[2] that probably it is a fresh-water form, which sometimes occurs also in brackish and sea-water, perhaps *Planaria torva* Müll. The American species *Fovia warreni* and *grisea* (littoralis[3]) spoken of by Verrill as *Fovia affinis* (Oe.) belong to only one species of the genus *Procerodes*, which must be designated *Procerodes warreni* (Gir.); the variations of color not being greater than usual in sea- and fresh-water-planarians, do not allow of forming separate varieties; the genus *Fovia* must be included in the genus *Procerodes*. Girard (4, 7, 8) described it as viviparous: the larvæ resemble the adult animal, but its anterior end is less truncated. Eyes are still absent in the larvæ, but the position of these is indicated by two transparent spots; a canal in the middle of the body is interpreted as the alimentary tube.

These "larvæ" are protozoans (*Hoplitophrya*), living in the cavity of the pharynx and in the intestine of triclads. M. Schultze[4] found them in *Procerodes* (*Planaria*) *ulvæ* of the Baltic Sea and described them under the name of *Opalina uncinata*. I myself found them in large numbers in *Procerodes segmentata*

[1] Stimpson, W., "Prodromus, etc., *Proc. Acad. N. Sc. Philadelphia*, 1857.

[2] Wilhelmi, J. "Über *Planaria affinis* Müller." *Bergens Museums Aarbog*, 1907, Nr. 4.

[3] I collected them at Woods Hole and neighborhood and at Massachusetts Bay, summer, 1907.

[4] Schultze, M., "Beiträge zur Naturgeschichte der Turbellarien," 1. Abtheilung, Greifswald, 1851.

(from the Bay of Naples, the Mediterranean and Black Sea), in *Procerodes ulvæ* (from the Baltic Sea) and also in the free-living North American marine triclads. They live principally in the cavity and the ramifications of the gut without damaging the host. The transparent spots called by Girard the first traces of the eyes, correspond to the organs of attachment and the alimentary tube answers to the nucleus of the *Hoplitophrya uncinata*.

With a detailed study of these Prof. M. M. Metcalf is occupied; at present he has given some notices on its excretory organs.[1]

I shall give in my monograph of the marine triclads an anatomical and histological description of these free-living North American marine triclads.

I append the list of the places on the east coast of North America, where these free-living triclads have been found, and indication of the corresponding literature.

Bay of Fundy, *Procerodes wheatlandi* (*ulvæ*) 1893 Verrill (12).

Eastport, Me., *P.* (*Fovia*) *warreni* 1893 Girard (8).

Grand Manan, N. Br., *P. wheatlandi* 1854 Girard (6).

Grand Manan, N. Br., *P.* (*Fovia*) *warreni* 1893 Verrill (12).

Casco Bay, Me., *P. wheatlandi* (*frequens, ulvæ*) 1873 and 1893 Verrill (10) (12).

Casco Bay, Me., *P.* (*Fovia*) *warreni* 1873 Verrill 1893 (10).

Casco Bay, Me., *P.* (*Fovia*) *warreni* (*grisea*) 1893 Verrill (12).

Cape Elizabeth, Me., *P.* (*Fovia*) *warreni* (*affinis*) 1873 Verrill (10).

Gloucester, Mass., *P. wheatlandi* (*ulvæ*) 1893 Verrill (12).

Manchester, Mass., *P. wheatlandi* 1850 and 1851 Girard (2) (5).

Beverly, Mass., *P.* (*Fovia*) *warreni* 1893 Verrill (12).

Chelsea Beach, Mass., *P.* (*Fovia*) *warreni* 1852 Girard (7).

Boston Harbor, Mass., *P.* (*Fovia*) *warreni* 1850 Girard (4).

Sandwich, Mass. (Cape Cod Bay), *P. wheatlandi* (*Gunda*) *segmontata* 1900–01 Curtis (1).

Woods Hole, Mass., *P.* (*Fovia*) *warreni* 1873–74 Verrill (10).

Woods Hole, Mass., *P. wheatlandi* (*ulvæ*) 1893 Verrill (12).

Vineyard Sound, *P. wheatlandi* (*frequens*) 1855 Verrill (10).

[1] Metcalf, M. M., "The Excretory Organs of *Opalina*," Part II., *Arch. f. Protistenkunde*, 10. Bd., 1907.

Newport, R. I., *P. wheatlandi* (*ulvæ*) 1893 Verrill (12).
Point Judith, R. I., *P. wheatlandi* (*frequens*) 1855 Leidy (9).
Watch Hill, R. I., *P.* (*Fovia*) *warreni* (*grisea*) 1872 Verrill (10).
New Haven, Conn., *P. wheatlandi* (*frequens*) 1873 Verrill (10).
New Haven, Conn., *P. wheatlandi* (*frequens, ulvæ*) 1893 Verrill (12).

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Girard, Ch.

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3. '50 A Brief Account of Fresh-water Planariæ of the United States. Ibid., 1850, p. 264.
4. '50 Observations upon Planarian Worms. Ibid., 1850, pp. 363–364.
5. '51 Di Planarien und Nemertinen Nordamerikas. Kellers und Tiedemanns Nordamerikanische Monatsberichte für Natur- und Heilkunde. Philadelphia 1851, 2 Bd., p. 4.
6. '54 in: W. Stimpson, Synopsis of the Marine Invertebrata of Grand Manan, or the Region about the Mouth of the Bay of Fundy, New Brunswick. Smithsonian Contrib. to Knowledge, 6 Bd., Washington, 1854, pp. 27, 28.
7. '54 Description of Two New Genera and Two New Species of Planaria. Proc. Boston Soc. N. H., Vol. 4, 1857–54 (1852), p. 211.
8. '93 Recherches sur les Planariés et Némertiens de l'Amérique du Nord. Ann. Sc. Nat., Vol., 15, 1893.

Leidy T.

9. '55 Contribution Towards a Knowledge of the Marine Invertebrate Fauna of the Coast of Rhode Island and New Jersey. Journ. Acad. N. Sc. New Ser., Vol. 3, 1855, p. 143.

Verrill, A. E.

10. '73 Report upon Invertebrate Animals of Vineyard Sound and the Adjacent Waters, with an Account of the Physiological Characters. United States Commission of Fish and Fisheries, Commission's Report for 1871 and 1872, Washington, 1873, pp. 325, 332, 477, 480, 488, 633.
11. '74 Results of Recent Dredging Expeditions on the East Coast of New England. Amer. Journ. Sc. and Arts, New Haven, 1874, Vol. 7, p. 132.
12. '93 Marine Planarians of New England. Trans. Conn. Acad., Vol. 8, 1893, pp. 118–127.

MARINE BIOLOGICAL LABORATORY,
WOODS HOLE, MASS., August, 1907.

A CONVENIENT PARAFFINE BATH FOR INDIVIDUAL USE.

J. F. McCLENDON.

Paraffine baths may be classified according to source of heat, into oil, gas and electric; and according to mode of regulation of temperature into fixed, regulated by attendant and self-regulating. In the self-regulating baths, the amount of heat generated may be variable or the loss of heat variable. In using oil and gas there is more danger from fire than in using electricity. Baths with a fixed temperature regulation can be used only in rooms of approximately constant temperature, and their readjustment by an attendant is inconvenient. Apparatus for controlling the amount of heat generated is too delicate and expensive to be put in the hands of all students. There only remains the electric bath, self-regulated by varying radiation of heat. Several forms of baths using this method have been used, and the form herein described differs from them chiefly in size, shape and material.

The bath consists of a rectangular concrete trough having inside measurements of 21.5 cm. in length, 11 cm. in width and 9.5 cm. in depth, and a general thickness of about 3.5 to 4 cm. In making these troughs I molded the inside with some old pieces of glass and left the glass in as a lining (in case this was not done the inside might be painted or left bare — the paraffine would not soak through so long as the outside is below its melting point). The trough is filled with paraffine of melting point one degree or more (3 in present instance) higher than that used in imbedding. The top is closed by a thin piece of wood in the center of which is a flush lamp socket with opening downward. Into this socket is screwed a four candle power "incandescent" lamp of the spherical type. The lamp reaches about to the bottom of the trough, while the socket projects upward above the top of the bath and is connected by small flexible wires or lamp cord to a 110-volt circuit. In the wooden top near the lamp are cut holes of suit-

able size and shape for sinking receptacles for imbedding dishes, paraffine pitchers, etc. For holding small vials I sunk larger ones through the wooden top and filled the space between the two with lubricating oil to prevent cooling by currents of air when the bath was opened. A flat tile would be useful to cover each side of the bath, avoiding the lamp socket.

The temperature regulation is more perfect than in any other small bath I have seen. The heat of the lamp melts the adjacent paraffine until the radiation equals the heat supplied, and convection currents keep the melted portion at constant temperature. When the temperature of the room rises, more of the paraffine in the trough melts and increases the radiating surface. When the temperature of the room falls, part of this paraffine congeals and decreases the radiating surface. The paraffine in the imbedding dishes is always kept melted unless they have been set too deep or too far from the light. The bath is intended to be fireproof, but if space is the determining factor it should be made of metal, in which case a higher candle power lamp should be used. The trough might be made of glazed earthenware with double side walls, the spaces between these walls opening downward and the outer walls projecting below the bottom of the bath, thus insuring an air space beneath.

ZOÖLOGICAL LABORATORY,
UNIVERSITY OF MISSOURI.

THE SPAWNING HABITS OF CHROSOMUS ERYTHROGASTER RAFINESQUE.*

BERTRAM G. SMITH.

While a student at the University of Michigan during the years 1904–1907 I acted as assistant in zoölogy and when accompanying Professor Jacob Reighard on field excursions had many opportunities to observe, under his guidance, the breeding habits of minnows and other fishes. This familiarity with the unpublished work of Professor Reighard made it possible for me to make the observations recorded below, which are published with his permission.

About the middle of May, 1907, I found the minnow *Chrosomus erythrogaster* Rafinesque very abundant in a small brook near Lake Forest, Ill. As the males were in breeding dress I seized the opportunity to make some observations on the spawning habits.

Habitat. — The brook flows through a large open pasture and also through woodland; the minnows were far more numerous in that portion of the brook which is in the open field. Here the stream meanders through a shallow valley, over a pebbly or sandy bottom. In places it is so narrow that one can readily step across it; elsewhere it expands into pools or shallow rapids not more than six feet wide. In the rapids the water is seldom more than two or three inches deep; in the pools it may reach a depth of one or two feet. The current is quite rapid; even in the pools there is little quiet water.

Chrosomus is by far the most abundant fish in this brook. Schools of from a dozen to several hundreds abound, while of other fishes only a few large suckers, and an occasional *Rhinichthys*, *Semotilus* and *Cottus*, were observed.

Sexual Dimorphism. — The bright colors of the males were scarcely noticeable when the fishes were in the water and viewed obliquely from above; but when removed from the water the

* Contributions from the Zoölogical Laboratory of Syracuse University.

males were found to be marked on each side of the abdomen with a broad longitudinal stripe of the most vivid and brilliant scarlet that I have ever seen. This stripe starts just back of the operculum and runs immediately below the lower of the two lateral dark bands (see Fig. 1) and parallel to it, reaching almost to the caudal fin. There is also a small red spot just below the

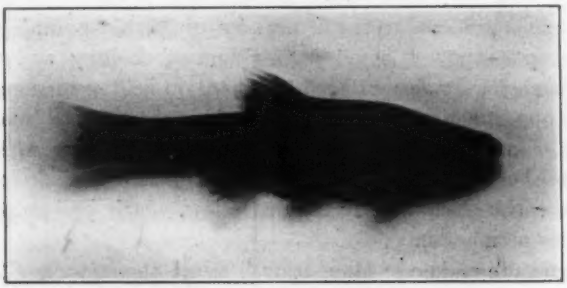


FIG. 1.

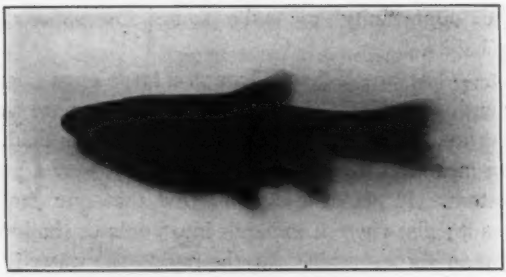


FIG. 2.

FIG. 1, male, and Fig. 2, female specimens of *Chrosomus erythrogaster* Rafinesque, life size.

mandible on each side. In some specimens the lateral bands of red are faint except just behind the operculum. In the more highly colored males the entire abdomen is covered with red, and there is a red spot in the anterior part of the root of the dorsal fin. The pectoral and pelvic fins, and the anal fin, are bright lemon yellow; the dorsal fin and tail are faintly marked with pale yellow. There is a small spot of yellow on the ventral side of the body at the base of the caudal fin, and another in the gular region.

The males possess "pearl organs" (small conical thickenings of the epidermis), occurring over practically the entire surface of the body, head, tail and fins. They are especially numerous and well developed on the dorsal and lateral surfaces of the body, on the opercula, over the entire surface of the tail, and on the dorsal surfaces of the pectoral fins; they are large, but comparatively few in number, on the dorsal surface of the head. They are much smaller on the ventral surface of the abdomen, and entirely lacking about the mouth and at the tips of the fins.

As seen under the microscope, a pearl organ from the body of a male is a sharply pointed transparent spine, rising from a broad base and pointing obliquely backward, occurring as a thickening of the epidermis over the middle of the posterior margin of each scale. Usually there is only a single pearl organ for each scale, but sometimes two spines occur close together. Smaller rudimentary pearl organs, each consisting of a papilla or thickening of the epidermis without a spine, also occur, one or more over each scale and usually near its center.

The pectoral fins of the male are much larger and stronger, in proportion to size of body, than those of the female. As shown by average measurements, the pelvic fins of the male are also proportionally larger than those of the female, but the difference is not so great as in the case of the pectoral fins.

The females are on the average slightly smaller than the males (see Figs. 1 and 2), but have the abdomen distinctly swollen. There is a small patch of red just back of the operculum. They have the same yellow coloration as the males, but the color is comparatively faint. They possess only rudimentary pearl organs, hardly visible except under the microscope.

A notable exception to the usual sexual dimorphism was found in the case of one unusually large female with well developed ovaries and eggs, but with the secondary sexual characteristics of the male all very well marked.

I had no opportunity to study the color changes and the history of the pearl organs throughout the year.

Sex Ratio. — In order to determine the numerical proportion of the sexes, representative samples of several schools were taken on several different days. The tabulated results are as follows:

Date of Capture.	Mode of Capture.	Number in Catch.	Females.	Males	Ratio.
1. May 17.....	Hoop net	6	2	4	1 : 2
2. " 29.....	Single sweep of seine	119	9	110	1 : 12.2
3. " 31.....	Hoop net	80	14	66	1 : 4.7
4. June 14.....	Hoop net	15	4	11	1 : 2.7
	Total..	220	29	191	1 : 6.5

In lots 1, 3 and 4 the sexes were determined by dissection. Since in only one of these specimens the secondary sexual characters were those of the opposite sex, the probable error in determining the sex by the external characters is a negligible quantity. In lot 2 the sexes were determined by the external characters.

These results are sufficient to indicate a decided preponderance in the number of males on the spawning ground at the spawning season, though not necessarily at other times or places.

Spawning Behavior. — The casual observer will find schools of *Chrosomus* occurring in the pools and the deeper portions of the swift water of the brook. On quietly approaching the stream, one will often notice a splashing of the water of the shallow rapids, scarcely distinguishable from the ordinary rippling of the current. Further observation will reveal the fact that in places the water is alive with tiny fishes, struggling and crowding one another in water so shallow that the suface is violently agitated; occasionally a mass of wriggling fishes will flash into view at the very surface, or crowd splashing upon the pebbly shore, where some may be left stranded, later to struggle back into the water.

When alarmed, the fishes almost invariably swim up stream from the shallows. In the deeper water they gradually recover from their fright; those that have hidden amongst the water vegetation or under the shadow of overhanging banks, rejoin the school. Together, moving in unison like a flock of sheep, they surge slowly down stream; but before they reach the shallow water again, they face about with an eddying movement, and swim up stream. This circuit is made repeatedly, the school drifting each time a little farther down stream toward their proper spawning grounds. The behavior of the fishes from the time they are frightened away from the shallows, until they resume

active spawning operations on the same ground, forms a series of events which occur on different occasions with unfailing regularity.

On the up stream journey, occasional isolated cases of spawning occur. Several males pursue one female; as the foremost males gain a position alongside the female, the flight and pursuit attain almost lightning-like rapidity. At length two males spawn with the single female as follows: One on each side presses the side of his head against that of the female, all three facing up stream. The two males then crowd laterally against the female, held between them (see Fig. 3); their entire flanks are thus pressed against the sides of the female. While the males are in this position, a rapid vibration of their bodies occurs. The wave of pressure begins at the anterior end of the body and passes backward as a sidewise undulating movement. Other males may attempt to crowd in. So far as observed, the female remains passive. The entire performance occurs so quickly that the details are made out only after considerable practice in observation.

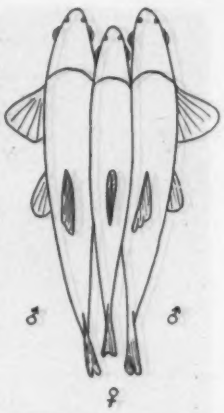


FIG. 3. *Chrosomus* in spawning position. Natural size.

Spawning under these conditions seems to be attended with difficulty. The act of clasping lasts but for an instant; the males seem unable to keep their position after the impetus of their rush up stream is overcome by the current, for swimming movements are necessarily discontinued during spawning. It is doubtful if spawning in the open, without contact with the bottom, is very effective; milt was not extruded in sufficient quantities to be seen. Although the eggs (almost transparent, with light-brown yolk, always difficult to see in the water) could not be seen, it was evident that some were extruded, for immediately after the spawning other fishes poked their noses into crevices between pebbles, just down stream from where the spawning occurred, evidently trying to get at the scattered eggs to eat them.

As the school in its circling movements nears the shallows, a

few of the foremost fishes, with shy, frightened movements, dart rapidly down stream through the swift water of the regular spawning grounds; after the first reconnoissance they hurry back to rejoin the main body, and the entire school moves up stream. This is repeated several times; each time the band of more venturesome fishes is increased in number, and a little later, on the up-stream journey, they stop for a time to spawn on the pebbly margin of the stream. Soon the entire school, in a compact body, is spawning on the shallows. With their swift movements

FIG. 4. Spawning grounds of *Chrosomus*. The shallow water near shore is occupied by a school of several hundred *Chrosomus;* some of these may be indistinctly seen, in spawning position, at the surface of the water in the center of the picture.

and brilliant colors, this writhing mass of several hundred fishes affords a truly remarkable spectacle.

Persistent attempts to photograph the spawning operations met with poor success, a result due in part to the lack of a suitable camera. Figs. 4 and 5 are reproductions of two of the many views taken.

This procedure of spawning *en masse* in shallow swift water

may be regarded as the normal spawning behavior of these fishes. All the fishes face up stream, but their swimming movements are only sufficient to keep them from being swept down stream by the current. In the confused and wriggling mass it is difficult to distinguish the movements of individual fishes; but it may be seen that many males crowd alongside a single female, and those immediately in contact with her may occasionally be seen in the spawning position described as occurring in the deep water.

Gradually the school crowds closer against the shore, rasping

FIG. 5. Spawning grounds of *Chrosomus*. Spawning fishes crowding up on the island of gravel, may be seen near the center of the picture. In the foreground the water is alive with fishes.

over the pebbles and wriggling into water so shallow that their dorsal surfaces are exposed. In these situations small groups become segregated from the main mass; their progress is impeded by the pebbles, so that accurate observation becomes an easy matter. Typically, two males lie alongside a single female; the group becomes wedged in between large pebbles and in close contact with the bottom, so that the males are enabled to keep

their position indefinitely, and lie for many minutes motionless except for the frequently occurring rapid vibration of their bodies. The spawning position is exactly the same as that observed in the open, but the performance is greatly prolonged, and spawning doubtless much more effective. Frequently, groups of four or even more fishes were seen lying alongside, with their bodies in close contact; occasionally a group of six, composed of two trios, each consisting of a single female, spawning with two males.

One marked variation from the usual spawning method was observed. A female in very shallow water spawned with a single male, which crowded her laterally against some pebbles and curved his tail up over her body, thus holding her firmly against the bottom. His body vibrated rapidly and the water became cloudy with milt.

Some further observations on the spawning behavior were obtained by the study of a small school which occupied an abandoned dace's nest. When first observed, this school consisted of a dozen or more males, crowded closely together in the hollow of the nest. Occasional females approached shyly from down stream, singly, or two or three in succession; sometimes darting rapidly, as if excited or frightened, up the middle of the stream; again keeping close to the shore, and occasionally seeking cover. The arrival of a female created intense excitement amongst the males in the nest. They immediately crowded around her, pressing alongside and against her in the hollow of the nest. After spawning, the males devoted considerable attention to prying about between the pebbles as if searching for eggs. Sometimes the males, in a body, left the nest and returned to it repeatedly, moving in an excited manner as if seeking to entice or drive a female into it. The nest was evidently an especially favorable place for spawning.

Eggs in various stages of development were obtained by scooping up the gravel of any of the spawning grounds.

Breeding Season. — The earliest observations were made on May 17. At this time the bright colors of the males were well developed and spawning was in progress, but how long it had been going on is not known. The latest observations were made on June 14; then the schools of fishes spawning on the shallows

were small, while in the pools below were larger schools which took no interest in spawning, and were comparatively inactive. The males of these latter schools had nearly lost their bright colors. Evidently the breeding season was nearly over. At this time many of the eggs had hatched out.

During this prolonged breeding season, extending over fully a month, the stream was frequently swollen by freshets, which presumably interfered with the spawning operations.

Discussion. — As shown by the unison of their circling movements when not engaged in spawning, and by the fact that they occur in schools even after the spawning season is over, these fishes possess a strong gregarious instinct. The two conspicuous longitudinal dark stripes on the sides of the bodies of both sexes probably serve as recognition marks.

The distribution of pearl organs over practically the entire body of the male affords a roughened surface which during spawning aids him in keeping his position beside the female. The function of the pearl organs of the Cyprinidæ was first discovered and described by Reighard ('03). The structure of pearl organs has been figured by various writers.

The greater size and strength of the pectoral fins of the male as compared with the female, and the fact that the dorsal surfaces of these fins are unusually well provided with pearl organs, indicate that these fins are of use to the male in holding the female. The male, coming up from the rear, doubtless interlocks his pectoral fin between the pectoral fin and the body of the female, though it was impossible to see this.

The excessive number of males present on the spawning grounds is perhaps correlated with the method of spawning, since two males spawn with a single female.

The observable factors in sex recognition are: the brilliant colors and aggressive bearing of the male; the dull colors, swollen abdomen, and timid, hesitating movements of the female.

It is significant, in its bearing on current theories of secondary sexual characteristics, that the female showing the secondary sexual characters of the male was an unusually large and apparently vigorous specimen; but it would be unwise to base conclusions on a single instance.

The total absence of combat amongst the males accords with the necessity for coöperation in the spawning act. Competition amongst the males is limited to the struggle to get a position next to the female; in this contest the swiftest and strongest succeed.

In general the method of spawning is adapted to fishes with gregarious habits, and would seem hardly likely to be developed amongst fishes whose usual habit of life is solitary.

The pebbles amongst which the eggs lodge afford them some protection, though it appears that some of the eggs are eaten by the parent fishes. The gradual subsidence of the water during the latter part of the season must have left some of the eggs to perish on the shore. That the method of spawning is, in general, a successful one is attested by the abundance of these fishes in this brook.

A comparison with the habits of other inland fishes would be impossible without reference to the unpublished work of Professor Jacob Reighard.

SYRACUSE UNIVERSITY,
April, 1908.

LITERATURE.

Reighard, Jacob.

'03 The Function of the Pearl Organs of the Cyprinidæ. Science, N.S., Vol. XVII., No. 431, p. 531.

THE PROCESS OF EGG-MAKING IN A TREMATODE.

EDWIN LINTON.

An outline of the process here described was published by me in connection with the description of the species *Epibdella bumpusii*, an ecto-parasite of the sting ray (*Dasyatis centrura*).[1]

Since opportunities of witnessing this instructive phenomenon are rare, and descriptions are wanting, even in the larger text-books, and, further, since the knowledge of the process has proved to be of great value in explaining to students the anatomy of the reproductive apparatus of the trematodes, I venture to publish a description of the process as I have seen it.

Every teacher of zoölogy is familiar with the anatomy of some typical trematode. The description of the relations of the various parts of the complicated reproductive machinery is about as far as one is apt to go in lecturing to a class, unless he has happened to see the machinery in operation, or, possibly, has had access to a description of the physiological working of that machinery. If what I have here written will help to vivify now and then a lecture on the reproductive apparatus of the trematodes I shall be repaid for the small trouble it has been to write out this narrative.

The species *E. bumpusii* is peculiarly well adapted for the study of the process of egg-making. The animal is small enough to allow of satisfactory study with low magnification, and is, withal, transparent enough to permit rather minute details to be clearly seen. Furthermore, the animal can be kept for several hours in sea water under slight pressure without impeding the normal action of the reproductive organs.

Anatomy.

In this description only the anatomy of the reproductive organs is considered. Reference to the figures and the explanation of the same will be necessary in order to follow this description.

As in most trematodes the individuals in this species are her-

[1] Bulletin U. S. Fish Commission for 1899, pp. 286-7, figs. 11-15.

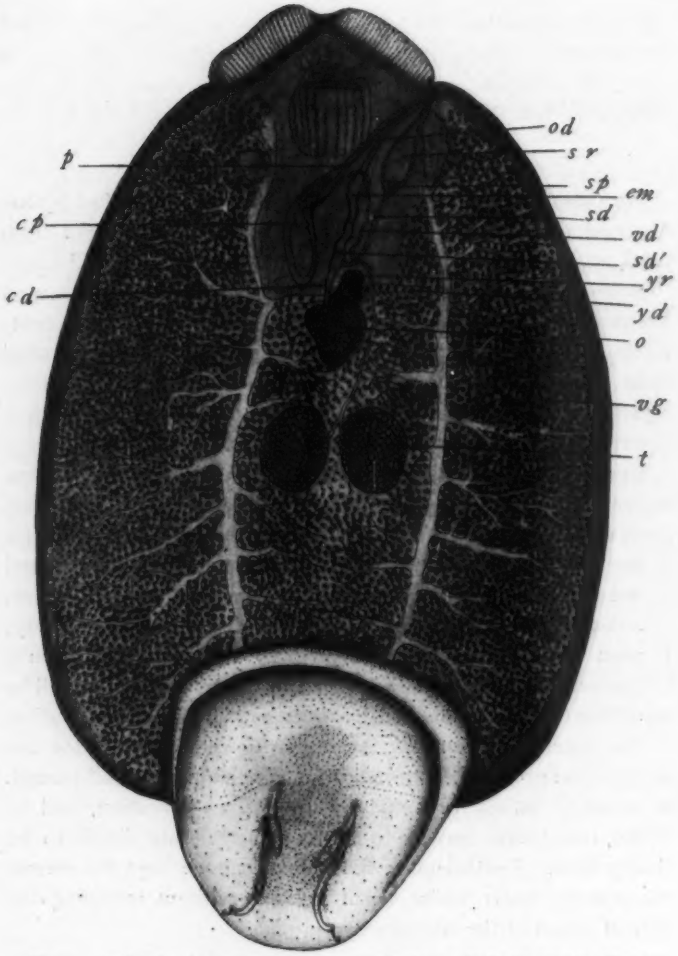


FIG. 1. *Epibdella bumpusii* Linton, ventral view; vitellaria partly diagrammatic. Actual diameter at level of testes 4.5 mm.

maphroditic. The male genitalia consist of an eversible cirrus (*p*), a cirrus-pouch (*cp*), in which is the seminal vesicle (*sv*). A vas-deferens (*vd*), which follows a somewhat tortuous course, may be easily traced from the seminal vesicle to the two testes

(*t*), which are subglobular in shape and lie near each other transversely placed and a little in front of the middle of the body. The cirrus pouch pulsated rhythmically and continued to pulsate long after all movement had ceased in the other organs.

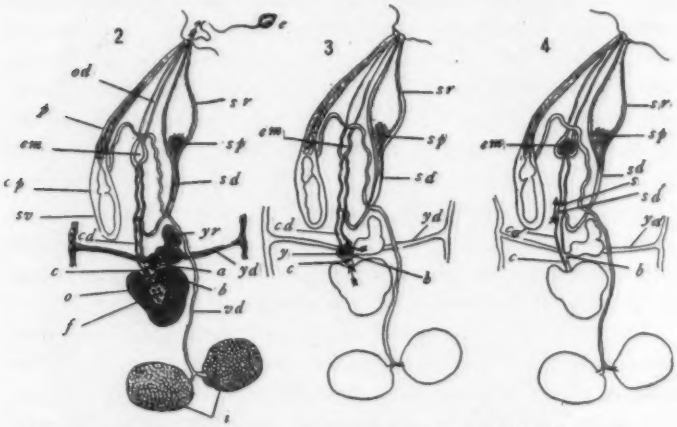
The female genitalia are more complicated. Filling the body laterally and posteriorly are the vitellaria (*vg*). These are dendritic glands whose ducts ultimately are collected into a transverse duct (*yd*) which enlarges into a vitelline reservoir (*yr*), lying a little to the left and a little in advance of the germarium (*o*). Immediately in front of the testes is the germarium (*o*), a somewhat trilobed organ lying on the median line. A short duct (*c*) passes at first dorsad then cephalad from the germarium where it joins another short duct (*b*) coming from the vitelline reservoir (*yr*). The common duct (*cd*) then passes near the median line, cephalad to the shell mold (oötype) (*em*), which lies near the cirrus-pouch and to its left. About midway between the germarium and the oötype the common duct is joined by a minute duct (*sd*, *sd'*), which enters on its dorsal side and comes from the seminal receptacle (*sr*). The seminal receptacle lies to the left and a little in advance of the oötype.

The cells which constitute the shell-forming gland surround the common duct for some distance back of the oötype. The oviduct passes forward from the oötype, lies beside the cirrus, and opens with it in the genital notch which lies on the anterior border on the left side of the head. The vagina opens in the genital notch close to the left side of the oviduct. It leads into a capacious seminal receptacle (*sr*), from the caudal end of which the seminal duct (*sd*), above mentioned, proceeds mediad to the common duct from the vitelline reservoir and germarium.

Physiological Action of the Genitalia which are Concerned in the Process of Egg-Making.

Following are the events named in order as they were seen to occur in a specimen which was lying in sea water under a coverglass. First, a lobe (*a*) of the vitelline reservoir (*yr*) contracted vigorously thereby emptying itself of a definite mass of the coarsely granular vitelline substance with which it was filled. As this takes place in a definite portion of the reservoir, it fol-

lows that approximately the same amount of yolk is thus discharged at each contraction. The mass of yolk is propelled with comparative rapidity along the short vitelline duct (*b*) towards the median line. This duct passes near the anterior border of the germarium (*o*), where it is joined by another short duct (*c*) from the germarium. As the mass of yolk was passing the germarium it was noticed that a number of free nucleated cells, which appeared to be lying loosely in a median area (*f*) of the germarium, were set in oscillatory motion. It was quite evident



FIGS. 2, 3, 4. Diagrams showing different stages of egg-making.

FIG. 2. An egg (*e*) has just been discharged from the oviduct (*od*). A mass of yolk for the next egg is collecting in a muscular lobe (*a*) of the yolk reservoir and is about to be ejected through the short duct (*b*) into the common duct (*cd*).

FIG. 3. The mass of yolk (*y*) has entered the common duct (*cd*) and its suction is probably an inciting cause of a germ's entering the common duct from the germ-duct (*a*). The direction of movement of yolk and germ is shown by the arrows.

FIG. 4. The mass of yolk has passed by way of the common duct to the oötype (*em*) where it has been molded into a tetrahedral shape and a shell has been deposited around it. A cluster of spermatozoa (*s*) has been injected into the common duct (*cd'*) from the muscular seminal duct (*sd'*) at its opening (*sd''*) and has proceeded a short way towards the oötype. The arrow shows the direction of movement of the spermatozoa. In the process of egg-making the egg is discharged from the oötype a very short time (one or two seconds) after the spermatozoa have arrived at the oötype.

that the agitation of the germ-cells was due to the passage of the mass of yolk from the vitelline reservoir. While it was

certain, from what could be made out from the mechanism of the living worm, that one result of the rush of yolk in passing the germarium (see arrow in Fig. 3 lying partly on the yolk reservoir), was to draw a germ-cell from the duct (*c*) (see arrow on germarium, Fig. 3), no cell was seen to leave its place in the germarium to enter the common duct (*cd*). The reason for this failure to see a germ-cell join the mass of yolk appeared when sections were studied. It was then seen (Fig. 5) that the germ-duct (*c*) leaves the germarium on its dorsal side, and is therefore seen only in end view when the worm is flattened out on the slide. In so much of the duct as was visible there were many free germ-cells, all of them oscillating more or less and thrown into vigorous agitation at the time when a mass of yolk was

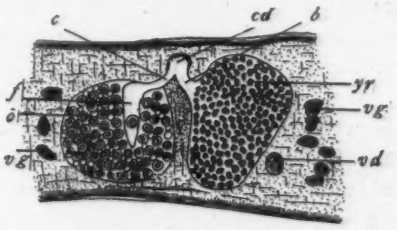


FIG. 5. Transverse section showing junction of ducts from yolk reservoir and germarium in common duct. Actual thickness of body through germarium 0.5 mm.

passing the germarium. If a cell were to leave the germarium its motion would be in a direction perpendicular to the eye, and therefore could not be detected. The portion of the duct which proceeds cephalad is somewhat concealed by the germarium. Hence here is one point in the process of egg-making, which, although perfectly obvious from the construction of the mechanism, was not actually seen. As the duct is at first perpendicular to the surface of the worm, it is obvious that the progressive motion of the germ-cell cannot be detected whether viewed from the dorsal or the ventral side.

The mass of coarsely granular yolk (Fig. 3, *y*) passes without pause and with comparative rapidity along the common duct (*cd*) to the oötype (*em*), which is simply a specialized part of the common duct. As soon as the mass of yolk reaches the oötype the passage closes by the approximation of the walls of the duct

thus forming a solid base on which the yolk rests. Against this base the mass of yolk is hammered by the walls of the oötype. Under this hammering the mass assumes a tetrahedral shape, and during the process the shell is built around it. The material for this shell is secreted by the gland which lies on each side of the common duct between the germarium and the oötype. When the shell is nearly finished, as shown by the cessation of the hammering process of the walls of the oötype, a very small and finely granular mass (Fig. 4, *s*) makes its appearance suddenly in the common duct at a point (*sd'*) approximately half way between the germarium and the oötype. This cluster of granules, which was in no case seen until it made its appearance in the common duct, travels rapidly along the common duct to the oötype. As soon as it reaches the oötype which now contains the newly encapsuled yolk mass with its associated germ-cell, there is a pause in the movements of the walls of the oötype and common duct for an instant. This pause is followed by powerful contractions of the walls of the oötype whereby the egg is forcibly ejected through the uterus (*od*) into the water.

The minute granular cluster which was seen entering the common duct immediately before the discharge of an egg, was interpreted at the time of observation to be spermatozoa, although the duct leading from the seminal receptacle to the common duct could not be seen in the living worm. It can be seen, however, in a stained specimen mounted in balsam and was satisfactorily demonstrated by means of serial sections.

Both the yolk mass and the spermatozoa appear to be propelled along the common duct by ciliary action. The egg is ejected by powerful muscular action of the walls of the oötype.

An examination of serial sections showed an interesting feature in the structure of the duct which leads from the germarium to the common duct. It is spacious at its beginning in the gland (*f*), where it appears in life as a clear space in the center of the germarium in which ripe germs could be seen oscillating whenever a charge of yolk was passing towards the oötype. The duct grows narrower distally. Indeed the duct (*c*) is shaped like a funnel. Near the point of union with the yolk duct (*b*) it is but little wider than the diameter of a single germ cell. A study of

sections makes it quite clear that the germ duct (*c*) and the yolk duct (*b*) connect in such manner that when a mass of yolk rushes along the yolk duct past the opening of the germ duct and into the common duct (Fig. 3, *b*, *c*, *y*, and arrow) sufficient suction is created to draw a germ from the germ duct.

Since the amount of yolk which is necessary for a single egg is automatically emptied into the yolk duct, the whole reflex has become adjusted with such marvellous nicety, the several parts to each other, that it seems probable that just enough suction is created to draw a single waiting germ cell from the germ duct which thus joins the passing charge of yolk.

The stimulus which causes a discharge from the seminal duct (*sd*, *sd'*) into the common duct always to take place immediately upon the completion of the process of molding the egg-shell is, of course, not obvious. The whole complicated process is an intricate nerve reflex.

Egg-making was observed to proceed actively for ten minutes or more to be followed by a short interval of rest. The time occupied in making an egg was not noted until the specimen had been under observation for two or three hours, by which time the worm had doubtless lost some of its vitality. When noted, the time from the moment at which the yolk left the reservoir until the completed egg was ejected into the water was about 40 seconds.

Summary of Events.

1. A mass of yolk leaves the yolk reservoir (*yr*).

2. As the yolk mass passes the germ duct (*c*) a germ is drawn out by the suction created by the moving mass of yolk.

3. The yolk mass and germ together pass along the common duct (*cd*) to the oötype (*em*).

4. An egg is molded into a tetrahedral shape by a kind of hammering action of the walls of the oötype, at the same time a shell is formed, its substance being secreted by the shell-forming gland.

5. A slowing up of the action of the oötype is followed by the appearance of a minute cluster of sperm in the common duct (*sd'*, *s*).

This cluster of sperms comes from the seminal duct and passes along the common duct to the oötype.

6. A momentary pause marks the arrival of the sperm at the oötype.

7. Powerful contractions of the walls of the oötype eject the egg from the uterus into the water.

Emendation of the Description of the Species.

In the original description of this species mention was made of but two pairs of hooks on the sucker. A third pair of minute hooks, posterior to the others, was subsequently found (Fig. 1).

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Explanation of Abbreviations.

Meaning of letters which are used in more than one figure.

b. Yolk duct leading from yolk reservoir to the common duct.
c. Germ duct leading from the germarium to the common duct.
cd. Common duct leading from the junction of *b* and *c* to oötype.
cp. Cirrus-pouch.
em. Oötype.
f. Beginning of germ duct in germarium.
o. Germarium.
od. Oviduct leading from oötype to exterior.
p. Cirrus.
sd. Seminal duct leading from seminal receptacle to common duct.
sd'. Point where *sd* enters the common duct.
sp. Spermatozoa in seminal receptacle.
sv. Pulsating organ at base of cirrus-pouch.
t. Testes.
vd. Vas deferens.
vg. Vitelline gland.
yd. Yolk duct leading from vitellaria to yolk reservoir.
yr. Yolk reservoir.

FORM REGULATION IN CERIANTHUS ÆSTUARII.

C. M. CHILD.

During 1905–6 it was my privilege to enjoy for several months the facilities afforded by the laboratory of the San Diego Marine Biological Association at La Jolla, California. During this time the work which forms the subject of the present paper, together with other work to appear later was accomplished. I take this opportunity of expressing my appreciation of the kindness of Professor W. E. Ritter and the other members of the association in granting me the privileges of the laboratory.

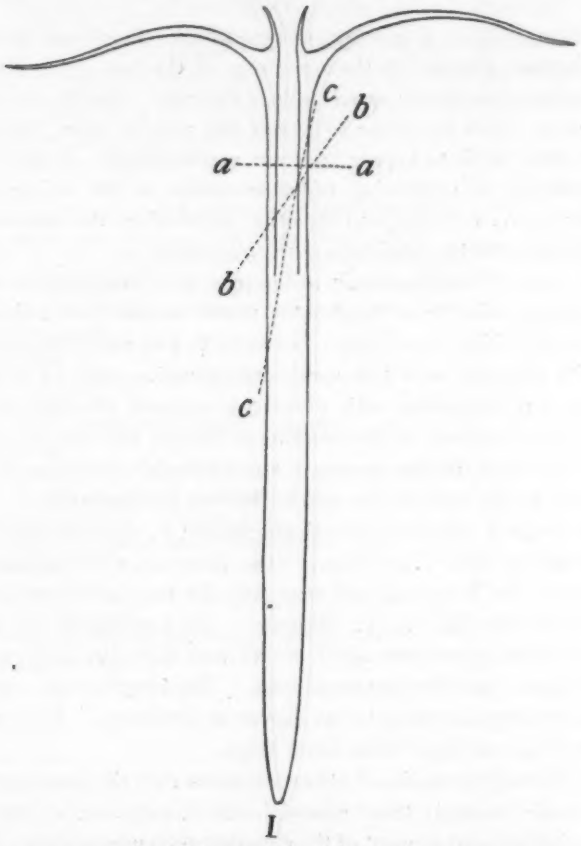
In 1902–3 I made a study of the process of form-regulation in *Cerianthus solitarius* at Naples, the results of which have already appeared (Child, '03*a*–'05*b*). In order to test and compare the results obtained with that species an extensive series of experiments was performed with *Cerianthus æstuarii* (Torrey) which occurs in abundance on the tide-flats of Mission Bay near La Jolla. For the name of the species I am indebted to Professor H. B. Torrey, as his work on the species has not yet appeared.

As regards size and general appearance *C. æstuarii* does not differ widely from *C. solitarius*. It is, however, more delicate in structure, the body-wall and especially the muscular layer being much thinner than in *C. solitarius*. In consequence of this characteristic specimens which are all well distended with water often appear more or less translucent. The length of the animal varies greatly according to the degree of distension. Fig. 1 will serve to give an idea of the usual shape.

In general the results of my experiments with this species confirm in all essentials those obtained with *C. solitarius*, so that an extended account of most of the experiments is unnecessary. But the results of experiment differ in some respects in the two species, and these differences, being due largely to the differences in the character of the tissues, possess a certain interest.

C. æstuarii, like *C. solitarius*, inhabits burrows whose walls consist of slime, nematocysts and fine sand or mud. Like *C. soli-*

tarius also it is capable of living for months in clear water in the laboratory, though, as will appear below, certain interesting modifications of shape and proportions appear under these conditions. The description of structure and habits given for *C. solitarius*



(Child, '03*a*, p. 239 et seq.) will apply in general to this species, but it may be noted here that *C. æstuarii* is more sensitive to external stimuli than *C. solitarius*. Slight contact-stimuli with needles or brush produce extreme and rapid contraction when the animal is normally distended with water, though it is apparently much less sensitive when partially or wholly collapsed.

Specimens normally distended are also very sensitive to light: if kept in covered dishes sudden exposure even to diffuse daylight produces marked general contraction after a short latent period. As regards this stimulus also collapsed specimens or pieces are much less sensitive than those which are distended with water.

The distension of the body and the erection of the tentacles is accomplished in this species as in *C. solitarius* by the entrance of water into the enteric cavity, both through the mouth and through the body-wall. The entrance of water through the body-wall probably occurs at all times to a greater or less extent, but is most readily observed in pieces undergoing regulation in which the ends have closed but no mouth has formed (cf. Child, '04*b*, p. 267 et seq.).

I. The Course of Form-Regulation.

In pieces above a certain minimal size, isolated by transverse cuts, the course of collapse and inrolling of the body-wall in the region of section, the closure of the wound and the formation of new tissue are in general similar to those processes in *C. solitarius* (Child, '03*a*, pp. 244–257, Figs. 1–24). As regards the formation of new tissue at the aboral end there is a quantitative difference between the two species, the amount of new tissue formed in *C. æstuarii* being usually much less than in *C. solitarius* (Child, '03*a*, pp. 257–259, Figs. 25–31). Attention may be recalled to the fact that the marginal tentacles in *C. solitarius* do not arise at the cut surface itself, but a short distance aboral to it (Child, '03*a*, p. 252, Figs. 10–19), the first indications of their formation being a reduction in the thickness of the body-wall in this region and the formation of a crenated ridge around the end of the piece, followed by the outgrowth of a tentacle above each interseptal chamber. These processes follow a similar course in *C. æstuarii.*

One difference between the two species may, however, be noted in this connection: in *C. æstuarii* the inrolling of the body-wall is much less regular than in *C. solitarius*, and closure in the usual manner is retarded or prevented more often in the latter species because the formation of the thin membrane closing the cut end is impossible. In consequence of the lack of firmness and stiffness in the body-wall of *C. æstuarii* the pieces often assume very

irregular forms when they collapse at the time of operation. Certain parts of the margin at the cut end may roll inward to a much greater extent than others, so that the cut surfaces on the two sides of the body may fail to approximate at all and closure cannot occur. The results of experiment are therefore less constant and uniform than in *C. solitarius*, though in general similar.

As regards the inrolling of cut margins in pieces of various form, longitudinal and transverse strips, oblique pieces, etc., all that has been said regarding *C. solitarius* (Child, '04*a*) will hold for this species. Here, in fact, results may vary even more widely than in *C. solitarius* because the body-wall is much more flaccid in this species after collapse. It is possible to inhibit completely the process of restitution simply by cutting the pieces of certain shapes so that the inrolling of the body-wall will prevent approximation of the cut margins: under these conditions closure of the opening and formation of the missing parts does not occur.

The regional differences in the power of regulation are very similar in the two species (cf. Child, '03*b*). The rapidity of oral form-regulation decreases with increasing distance of the level of section from the oral end.

As regards the relations between size of the piece and regulation the same similarity between the two species obtains. The length of tentacles is distinctly not proportional to the size of the piece (cf. Child, '03*b*, Part II., pp. 3–6). In *C. æstuarii* the uncertainty as to the size of minimal pieces is still greater than in *C. solitarius*, because of the great irregularity in the process of inrolling in short pieces.

In neither species has the outgrowth of new tissue directly from a free cut surface ever been observed (Child, '04*a*, pp. 66–74, Figs. 25–31; '04*b*, pp. 276–279, Figs. 3 and 4). On a free cut surface of the body-wall exposed to the water scarcely a trace of growth of new tissue appears and no restitution of the missing parts. When, however, two cut surfaces or two parts of a cut surface, *e. g.*, the two margins of a fold at the cut end, come into contact fusion occurs between them, but no further growth takes place unless the region is subjected to tension produced by water in the enteron or in some other way. But when the region is subjected to tension proliferation and growth occur

and a thin membrane of new tissue is formed, whose size is dependent in part on the degree of tension existing. When pieces are cut in such shapes, *e. g.*, by longitudinal or zig-zag cuts, etc., that the cut margins or parts of them do not come into contact with each other, the outgrowth of new tissue does not in any case occur from the free cut surfaces. Such pieces may remain open indefinitely and fail completely to replace the parts removed.

In many cases after the growth of new tissue has begun between approximated parts of the cut surface the new tissue may gradually extend for a very short distance over the cut surface on each side of the point where it began to grow. In such cases it can readily be determined that the thin new tissue is itself under some slight degree of tension. Under these conditions growth proceeds to a certain point and then stops. Apparently the mechanical conditions in the region involved and the physical properties of the new tissue itself are important factors in determining the amount of growth. In my earlier experiments the fact was noted

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that such growth occurs to a much greater extent in *C. membranaceus* where the new tissue is much thicker and more resistant than in *C. solitarius* (Child, '04*a*, pp. 70–71). In *C. æstuarii*, where the tissues are even more delicate such growth of new tissue does not occur to any great extent. In Fig. 2 the end of a piece cut so that it remains widely open is shown and the extent of the growth of new tissue is indicated by the shaded areas in the angles of the inrolled margins. Such a piece never closes by extension of the new tissue over the whole opening, but remains indefinitely in the condition figured, unless the relation of the parts of the cut margin is altered by changes in the degree of contraction or by passive changes of the position ofthe piece, which bring new portions of the cut margins into contact or proximity. After such a change growth of new tissue may continue for a time where the angle between two adjoining parts of the cut margin is not too great, but in no case does the new tissue extend with

a free margin across wide spaces as in *C. membranaceus* (Child, '04*a*, Fig. 31).

If the piece becomes distended with water after closure of a cut end by new tissue the new tissue, which at first is scarcely visible between the margins of the old parts, increases in area, but in the absence of distension such growth never occurs. Fig. 3 on p. 277 of my earlier paper (Child, '04*b*) shows the growth of new tissue in a collapsed piece of *C. solitarius* and Fig. 4 the growth in a distended piece. The differences in *C. æstuarii* are similar.

There is then no escape from the conclusion that mechanical strain is a necessary factor in the growth of new tissue in these species. That it is the only factor, I should be the last to assert, but I think it is sufficiently clear that in its absence the *Cerianthus* material possesses no inherent power to restore missing parts.

II. The Control of Tentacle-Development in Regulation.

(*a*) *By Artificial Openings in the Body-Wall.*

After the closure by new tissue of the ends of pieces distension of the enteric cavity with water occurs before any opening into the enteron exists. The passage of the water through the body-wall is probably not a simple osmotic process but something more complex. When the mouth is present or after a new mouth is formed water may enter through it, and by these means a certain degree of distension is maintained. In my experiments with *C. solitarius* it was possible to prevent, at least in large measure, this distension by making openings in the body-wall and reopening them at short intervals (Child, '04*c*). Under these conditions the development of the tentacles is retarded or almost entirely inhibited.

The secretion of slime about the opening and the inrolling of the margins of the cut make it impossible to prevent all distension, but by removing the slime and reopening the pieces every few hours or even once a day the distension can be reduced far below the normal and the first appearance and the rapidity of development of the tentacles greatly retarded and their final size much reduced. If it were possible to eliminate all internal pressure the tentacles would undoubtedly fail to appear.

Experiments along this line on *C. æstuarii* confirm my earlier work. It was possible to control the development of the tentacle in this species within wide limits: in some experiments, for example, they attained a length of 1 mm. in the open pieces while in the controls they reached the length of 15 mm. in the same time.

(*b*) *Pieces from the Œsophageal Region.*

The œsophagus in *Cerianthus* extends a considerable distance aborally from the mouth (Fig. 1), and pieces in which the œsophagus extends through the whole length can readily be obtained. The process of closure of the cut ends in such pieces was described for *C. solitarius* in my earlier paper (Child, '04*d*, pp. 205–206, Figs. 7–15), and is similar in *C. æstuarii*. In almost all cases the cut ends of œsophagus and body-wall unite at both ends of the piece and the œsophagus therefore opens to the exterior at each end but does not communicate with the enteron, at all. In these pieces water can pass into the enteron only through the body-wall, but distension by this means does not continue indefinitely (Child, '04*d*, pp. 206, 211) and after a few days the pieces gradually collapse and never become distended again. Under these conditions the development of the tentacles begins but ceases as the distension decreases, and later, when the piece becomes completely collapsed, the tentacles undergo atrophy (Child, '04*d*, pp. 207–212). The results of experiments of this kind on *C. æstuarii* are even more striking, for the atrophy occurs more rapidly. The records for two series of experiments are given by way of illustration.

Series 14.

August 30, 1905. — I. A piece with oral end about the middle of the œsophageal region (*a*, Fig. 3) and aboral end just distal to the aboral end of the œsophagus (*b*, Fig. 3).

II. As control a piece from another animal with oral end as nearly as possible at the same level as that of I., and with aboral end just proximal to the aboral end of the œsophagus (*c*, Fig. 3).

The difference in length of the pieces is very slight and both are far above the minimal size of pieces capable of complete restitution.

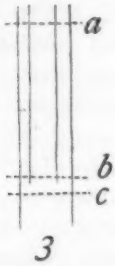
September 3. — I. Body-wall and œsophagus united at both

ends; piece somewhat distended with water; marginal tentacles just beginning to appear, about 1 mm. in length; no labial tentacles (Fig. 4).

II. Body-wall and œsophagus united orally so that a mouth is formed. Aborally the cut end of the body-wall is closed. The œsophagus therefore opens into the enteron. The piece is distended and the marginal tentacles are 2 mm. in length. No labial tentacles (Fig. 5).

September 9. — I. Piece slightly distended; marginal tentacles barely visible; no labial tentacles.

II. Fully distended; marginal tentacles 5–7 mm. in length; labial tentacles just visible.



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September 18. — I. Collapsed; neither marginal nor labial tentacles visible.

II. Fully distended; marginal tentacles 8–10 mm. in length; labial tentacles 1–2 mm.

October 8. — I. Collapsed; much reduced in size and body-wall undergoing atrophy. No traces of tentacles.

II. In burrow, distended; marginal tentacles 18–20 mm.; labial tentacles 4–5 mm.

Later History. — I. After October 8 complete atrophy of the body-wall along the line of folds occurred and the piece broke up into fragments which continued to decrease in size and undergo atrophy until they died.

II. Remained in good condition in burrow until December 31 when experiment concluded. Marginal tentacles 20–25 mm. in length; labial tentacles 5 mm.

The difference in the process of regulation between these two pieces is very great. In piece I. the development of marginal

tentacles began during the period of temporary distention, but after collapse these tentacles atrophied and no trace of labial tentacles ever appeared. Moreover the piece gradually underwent decrease in size and atrophy and finally broke up and died.

Piece II., on the other hand, gave rise to a normal animal with both marginal and labial tentacles and remained in good condition for months.

Series 35.

September 15, 1905. — Four œsophageal pieces from different individuals, each including about the middle half of the œsophageal region.

September 20. — In all the œsophagus and body-wall have united at both ends and the enteron is without communication with the exterior. The pieces are slightly distended and one shows slight elevations of the body-wall in the marginal tentacle region; the others are without any trace of marginal tentacles, and none show any trace of labial tentacles.

October 2. — All collapsed and reduced in size. No traces of tentacles in any. In three pieces the body-wall has atrophied and split along the line of longitudinal folds.

Later History. — All four pieces gradually atrophied and broke up and the fragments died. No further traces of tentacles appeared at any time. During the same time other pieces of the same and smaller size but with aboral ends proximal to the œsophageal region, closed, became distended, gave rise to marginal and labial tentacles and remained in good condition until the conclusion of the experiment on December 31, 1905.

In general the development of marginal tentacles is almost completely inhibited in œsophageal pieces and the tentacles which do appear undergo complete atrophy later. Labial tentacles have never been seen in the pieces. Moreover the pieces are incapable of continued existence for more than a few weeks in the absence of internal pressure.

(*c*) *The Development of Tentacles on Oblique Oral Ends.*

In *C. solitarius* the formation of tentacles on oblique oral ends is not simultaneous about the whole margin as it is on transverse ends, but the tentacles appear earliest on the most distal portion

of the oblique surface and latest on the most proximal portion (Child, '04*d*, pp. 193–205, Figs. 1–6). At least a part of this difference is due to the difference in level of the most distal and most proximal portions of the oblique surface, but in the paper referred to it was shown that the marginal tentacles appear earlier on the most distal portion of an oblique end and later on the most proximal portion than on transverse ends at these two different levels. These and other results led me to suggest that perhaps the currents which pass orally along the inner surface of the body-wall in each interseptal chamber might, as well as the general internal pressure, be a factor in tentacle-development (Child, '04*d*, pp. 193–205).

The results of experiment on oblique pieces in *C. æstuarii* are similar: part of the records for one series is given here.

Series 33.

September 15, 1905. — I. The oral ends were removed from four specimens by oblique cuts through the œsophageal region (*bb*, Fig. 1).

II. The oral ends were removed from four other specimens by transverse cuts as nearly as possible at the same level as the most distal portion of the oblique cuts (*aa*, Fig. 1). No control was made for the lower level of the oblique surface because the rapidity of tentacle-formation on transverse surfaces is not very different at these two levels. The history of one of the oblique pieces and one of the controls is given.

September 17. — The cut ends in the oblique pieces I. and the control II. were closed: tentacles were not visible in either, but the body-wall was becoming thinner in the most distal portion of the oblique piece (Fig. 6, diagrammatic longitudinal section).

September 20. — I. Marginal tentacles about 2 mm. long on the most distal portion of the oblique surface and decreasing in length from this point to zero about half way between the extreme distal and proximal portions of the disc. The most proximal portion of the disc shows no preparation for tentacle-formation as yet. Fig. 7 is a diagrammatic longitudinal section and Fig. 8 shows one side of the disc.

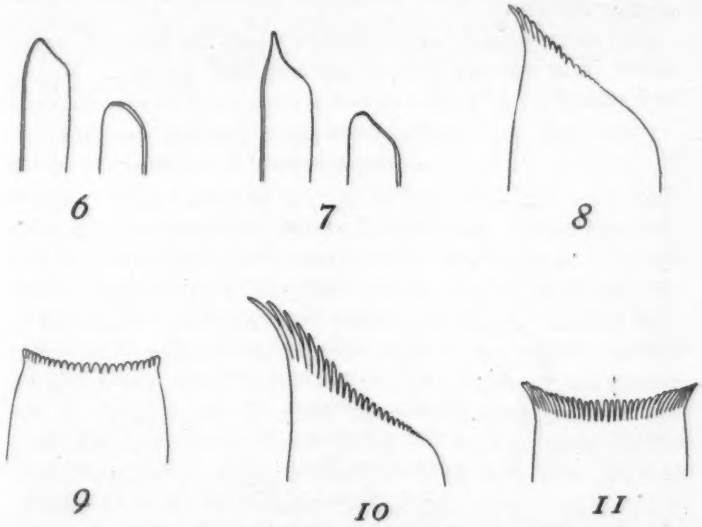
II. Marginal tentacles just appearing about the whole margin of the disc: 0.5–1 mm. in length (Fig. 9).

September 23. — I. Marginal tentacles on most distal portion 5–6 mm. in length, decreasing to slight elevations on the opposite side (Fig. 10).

II. Marginal tentacles 2–3 mm. in length about the whole margin (Fig. 11).

Later the tentacles on the oblique piece underwent a gradual equalization and the disc lost its obliquity as in oblique pieces of *C. solitarius*.

This series shows very clearly, and my other series afford similar results, that tentacle-formation is accelerated on the more

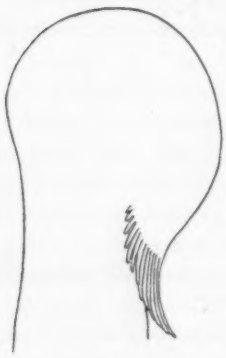


distal portions and retarded on the more proximal portions of the oblique piece as compared with transverse pieces.

Since internal pressure is in general so important a factor in tentacle-formation in *Cerianthus* it seems not at all improbable that the differences between the oblique and transverse pieces may be due to local differences in the internal pressure in the oblique pieces. The currents passing orally in each interseptal chamber enter the inrolled portion of the oral end and, as was suggested in my earlier paper, local differences in the internal pressure may be produced by these currents since the body-wall is always

folded over much more sharply on the more distal portions of the oblique end than on the more proximal. At all events the obliquity of the disc affects the rapidity of tentacle-formation in some manner, and the suggestion that local differences in internal pressure resulting from the different relation between the internal currents and the body-wall on different parts of the oblique end are concerned in producing the result observed seems at present to account for the facts. The question as to whether localized internal pressure is a factor in the localization of tentacles will be considered elsewhere in connection with certain experiments on another actinian.

One other series of experiments in which the plane of section of the body was very oblique deserves brief mention. In this series the plane of section extended from a region near the original tentacles on one side to a point considerably below the proximal end of the œsophagus on the other (*cc*, Fig. 1). The margin of the body-wall and the œsophagus united down to the proximal end of œsophagus, but since the cut extended a considerable distance beyond this point, there remained an opening of considerable size, which could be closed only by approximation of the margins of the body-wall and the formation of new tissue between them: this, however, occurred very slowly. The result of the operation was then at first a piece in which one side of the body-wall in the œsophageal region and to a point some distance proximal to it, and one side of the œsophagus were removed.



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The distal portions of these pieces underwent considerable decrease in length so that the obliquity of the oral end was reduced. In earlier stages distension with water was impossible because of the opening below the œsophagus, and tentacle-formation was much delayed. Later the most distal portion bent over and together with the slime secretion effected a provisional closure (Fig. 12), and some degree of distension took place and was fol-

lowed by the formation of tentacles. Below the œsophagus no tentacles formed because here no closure of the wound took place.

A month after the operation the pieces possessed the form shown in Fig. 12. The most distal part of the oral end was bent over so that the oblique disc was applied to the side of the body in the region of the opening below the œsophagus. The disc bore a semicircle of marginal tentacles which showed the usual differences in length of tentacles.

These pieces are of interest because they show an extreme type of reaction to the wound, but even in this case the reaction seems to consist essentially of contraction of the cut edges of the body-wall. The fact that this contraction produces so marked a change in the position of the oral end is due merely to the position of the cut.

III. Experimental Change in Length and Atrophy of Tentacles.

The marginal tentacles are 40–50 mm. in length when fully distended and the labial tentacles about 10 mm. Exact measurements are of course out of the question. Reduction and atrophy of the tentacles can be induced in this species as in *C. solitarius* by preventing distension of the body and tentacles. As has been noted above, openings in the body-wall do not entirely prevent distension, even when frequently reopened, for provisional closure occurs, sometimes within a few minutes after reopening. These provisional closures, however, cannot withstand anything like the normal internal pressure, so that even when they occur the total distension is very much below the normal.

In one series of this kind the tentacles were reduced in fifty days from full length to mere stumps, the marginal tentacles being 2 mm. in length and the labials just visible as slight elevations. Atrophy of the tentacles proceeds in the centripetal direction as in *C. solitarius*, and at the tip of each tentacle a small mass of degenerating tissue is visible (cf. Child, '04*e*, Figs. 3–5). If the specimens are allowed to close and become distended after atrophy of the tentacles, growth of the tentacles begins anew and proceeds according to the degree of distension established.

Tentacle-atrophy is a characteristic feature of pieces taken

entirely from the œsophageal region, in which the œsophagus and body-wall unite at both ends and there is no communication between the enteron and the exterior. The distension which occurs during the first few days after closure disappears later and complete collapse takes place. Under these conditions atrophy of the tentacles is very rapid: in some cases complete disappearance of fully developed normal tentacles occurred within thirty days. Tentacle-atrophy is always followed in these pieces by atrophy of the body-wall and finally fragmentation and death of the pieces.

Partial tentacle-atrophy can be induced in this species merely by keeping the animals in dishes without sand in which they can burrow. Without the support of the wall of the burrow the body-wall is unable to sustain the normal degree of internal pressure and the tentacles undergo partial atrophy in consequence of the altered conditions. Apparently the internal pressure is regulated to a greater or less extent by the strain upon the body-wall, for it is certain that in animals outside the burrows the internal pressure is not nearly as great as when the body-wall is supported by the wall of the burrow. So far as I can determine this difference is not due merely to escape of the water through the aboral pore when a certain degree of pressure is reached, for the pressure is always much below the point when opening of the aboral pore occurs: there seems rather to be a regulation of the entrance of water. Occasionally for some reason regulation does not occur or is insufficient and rupture of the body-wall takes place, usually in the aboral third, where the body-wall is thinner than in other regions.

After animals have been kept for five or six weeks under these conditions the marginal tentacles are usually 10–15 mm. and the labials about 5 mm. in length.

A marked difference in the length of tentacles produced in regulation likewise appears according as the animals are allowed to burrow or are kept without sand. Tentacles developing on pieces which live in burrows may attain the length of the original tentacles while those on pieces without sand reach only half this length or less.

The body-wall of *C. solitarius* is much thicker and more resist-

ant than that of *C. æstuarii* and is capable of supporting without the aid of the tube, a degree of internal pressure which closely approaches or perhaps equals the normal. In that species, therefore no marked reduction of the tentacles is observed in specimens kept without sand.

IV. Lateral Partial Discs.

The formation of lateral partial discs occurs in connection with lateral incisions and usually in the œsophageal region only, where the cut edges of the body-wall and the œsophagus fuse both above and below the cut and so give rise to a lateral mouth (Child, '05*a*). In all cases observed in *C. solitarius* the region between the lateral cut and the oral end of the body undergoes complete atrophy sooner or later and the lateral partial disc takes the place of the atrophied part. Atrophy occurs in this region because its enteric cavity has no connection either with the other portions of the enteron or with the exterior. After closure some distension occurs but collapse follows and continues until the part undergoes complete atrophy. Meanwhile the partial disc gradually changes its position toward the oral end and finally replaces the atrophied part.

(*a*) *Transverse Lateral Incisions.*

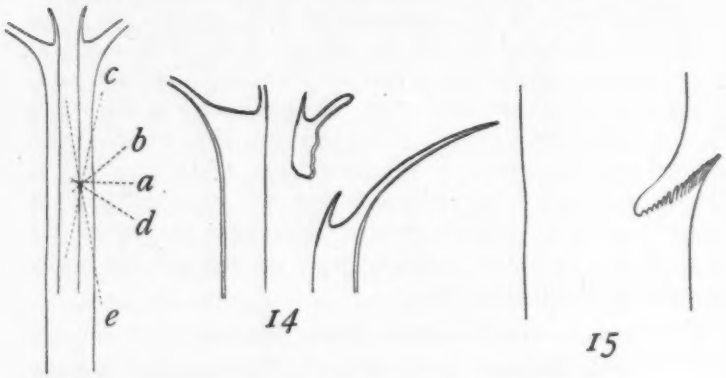
The only difference worthy of note in this connection between *C. solitarius* and *C. æstuarii* is the more rapid atrophy of the portion oral to the cut in the latter species. Frequently this atrophy is so rapid that breaks occur in the piece within a week or ten days after the operation, while in *C. solitarius* this condition is reached only after several weeks. Fig. 14 shows a longitudinal section of a specimen with lateral partial disc and the collapsed portion undergoing atrophy oral to it. The incision in this case was made at *a*, Fig. 13.

(*b*) *Oblique Lateral Incisions Directed Aborally.*

No oblique lateral incisions were made in my earlier work on *C. solitarius*, but a considerable number of operations of this kind have been made upon *C. æstuarii*. When the incision is directed aborally at any angle with the transverse plane up to 50°–60° (*b*, Fig. 13) partial discs appear which show the difference in

rapidity of tentacle-development of different parts of the disc characteristic of oblique discs (cf. pp. 35–38), the tentacles appearing earliest on the most distal portion and latest on the most proximal portion of the disc. A case of this sort is shown in Fig. 15. The later history of these oblique discs is similar to that of partial discs formed after transverse incisions, and during the change in position of the disc the obliquity gradually disappears as in other oblique discs and the result is an animal of usual shape.

But when the angle between the incision and the transverse plane is greater than 50°–60° (*c*, Fig. 13) the results differ from



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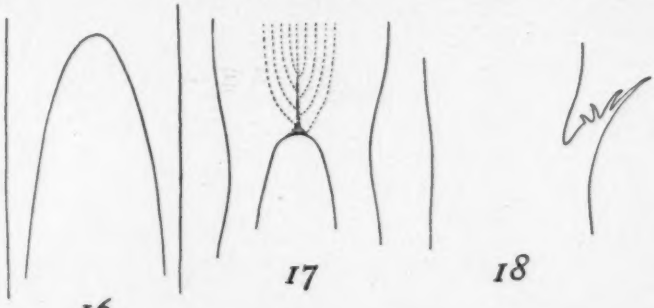
those described and in some cases permanent lateral discs are formed borne on a small column which arises as a branch from the side of the original body. As the plane of the incision approaches the longitudinal axis of the body the incision approaches a longitudinal direction and the process of wound-closure approaches that occurring after a longitudinal incision. The course of an oblique incision approaching the longitudinal direction is indicated in Fig. 16. After an incision of this kind the wound in the body-wall closes longitudinally by approximation and union of the cut margins (Fig. 17), but the oblique slip of tissue which was separated distally from the body-wall but is still connected with it proximally is not included in the closure and its cut margins also close longitudinally and independently of the

other part except at its proximal end where it is continuous with the rest of the body-wall. Thus the closure of the wound results in the formation of a small component extending obliquely in the distal direction from the side of the body. The œsophagus also closes longitudinally in each part and in the lateral component unites distally with the body-wall so that this component possesses a mouth. In this manner an oblique lateral disc is established at the distal end of the lateral component, but it is a complete not a partial disc, because the closure has been largely longitudinal. On such discs the tentacles arise in the manner characteristic for oblique discs, *i. e.*, earliest on the most distal portion and latest on the proximal portion of the disc (Fig. 18).



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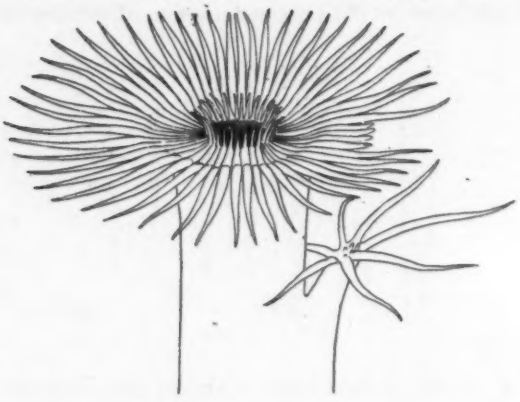
In Fig. 19 one of these cases is shown after complete development of the tentacles on the lateral disc, but before the obliquity has disappeared. In this figure it is seen that several of the tentacles on the original terminal disc, viz., some of those which were situated directly distal to the incision, have undergone partial atrophy. The stage figured does not, however, represent the greatest degree of atrophy which occurred. After the closure of the wound these tentacles underwent rapid atrophy until they were reduced to less than half the length shown in the figure and some of them were mere stumps 2–3 mm. in length. Then they began to grow again and would undoubtedly have finally attained the same length as the others if it had been possible to continue observation of the specimen for a longer time. The reason for the peculiar history of these tentacles is to be found in the method of closure of the wound. In Fig. 17 the direc-

tion of the septa in the region of the incision is indicated diagramatically by the dotted lines. With the approximation of the oblique margins of the cut the cut ends of the septa are also closely approximated, and since the œsophagus closes in the same manner as the body-wall the consequence is that some of the interseptal chambers distal to the apical region of the cut become almost or completely closed aborally, while lower down, where more new tissue is formed between the old margins of the body-wall they remain more widely open. When the body is distended with water those chambers which are nearly or quite closed aborally remain almost or wholly collapsed, since they



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are nearly or quite isolated from the other parts of the enteron, and the pressure resulting from the more rapid distension of other parts aids still further in closing small openings which may remain. After the distension resulting from the passage of water through the body-wall or septa has subsided this region remains collapsed and the tentacles corresponding to those chambers begin to atrophy. But in the further course of regulation after closure the area of new tissue increases and the old margins of the cut, now united by a thin membrane of new tissue, gradually separate as it increases, and the relations of the septa also undergo regulation in consequence. At a certain stage the interseptal chambers which were shut off from other parts of the

enteric cavity again become open and fill with water and renewed distension of the tentacles and consequently renewed growth occurs.

But the most interesting feature of these cases is that, so far as my observations go, the lateral component is permanent and does not undergo further regulation ending in union with the parts of the terminal disc which remain, as do the transverse and less oblique lateral discs. Some specimens were kept under observation during four months and at the end of this time there was no indication of any further regulation leading to fusion of the lateral component with the rest of the body. Fig. 19 is drawn from one of these specimens four months after the operation. This length of time is more than sufficient for the migration of the lateral disc to the oral end of the whole if it were to occur, but there is no evidence of any such change in position. Each component maintains its form and relations to the other. The only changes noted in later stages were, the increase in length of the lateral component, which would probably in time have reached the level of the original disc, the gradual reduction of the obliquity of the disc of the lateral component and new growth of the previously atrophied old tentacles above the cut.

The reason for the failure of the lateral disc to migrate to the oral end of the body and take the place of a part of the original disc lies in the simple fact that no part of the region distal to the cut underwent complete atrophy. Atrophy began, but the regulatory process involved in the filling of the area of the cut with new tissue brought about the opening and renewed distension of the interseptal chambers in this region and so determined its persistence. Evidently the presence of active non-atrophied parts distal to the lateral component prevents its migration to the oral end.

These cases are of considerable interest in that they indicate very clearly the dependence of "normal" proportions and positions of parts upon certain factors which are subject to experimental control, viz., in this case the direction of the incision and the distension or absence of distension of certain parts with water and their consequent persistence or atrophy.

(c) Oblique Lateral Incisions Directed Orally.

When the oblique incision is directed orally the results differ, according to the angle as in the case of aborally directed incisions described in the preceding section. If the angle between the incision and the transverse plane is not greater than 30–40 (*d*, Fig. 13) a lateral partial disc with mouth is usually formed, the parts distal to it atrophy and it gradually migrates to the oral end of the body. The sequence of tentacle-formation in these partial discs is that characteristic of other oblique discs, the tentacles appearing earliest on the two opposite ends of the partial disc, which in these cases are the most distal portions, and latest on the middle, most proximal portion.

When the orally directed incision is more oblique, however (*e*, Fig. 13) other results are obtained. In most cases of this sort complete closure of the wound occurred sooner or later without the formation of a lateral mouth or partial disc, *i. e.*, the cut margins of the body-wall united longitudinally with each other and not with the œsophagus, and the oblique slip, which in these cases is directed aborally, formed a small shrivelled excrescence, which underwent gradual atrophy and resorption.

After these incisions the margins of the body-wall approximate and unite longitudinally from the proximal end of the cut distally (Fig. 20, the area of new tissue is indicated by shading). When the approximation of the margins and the accumulation of slime has brought about a provisional closure and partial distension is possible, the more distal portions of the cut margin are pressed against the aborally directed slip which overlaps this region and union readily occurs here, so that the whole wound is closed without the formation of a disc. Here the result, in itself different from that obtained with aborally directed oblique incisions, is brought about by the same internal factors, *i. e.*, the characteristic reactions of the species, but the conditions under which the reactions occur are different, hence the difference in result. In the case of aborally directed incisions the partial distension separates the oblique slip from the rest of the body-wall, since the slip shares more fully in the distension than the portion of the body-wall directly distal to the cut. In the present case, on the other hand, the oblique slip is less distended than other

parts, hence it contracts and the other parts are pressed against it, permitting union of the cut margins to occur.

In several cases of this sort the incisions were reopened one or more times after they had healed in the manner above described, and in one case tentacles were produced, but later disappeared. The history of this case is briefly given since it presents some features of importance.

Series 31—III.

September 1. — Very oblique incision directed orally.

September 9. — Almost entirely healed; reopened.

Marginal tentacles on original disc 12–15 mm. in length except

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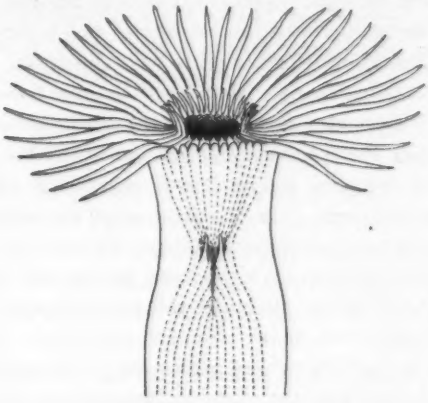

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above cut, where they are reduced to about 1 mm. Labial tentacles 3–4 mm., except over cut, where 1–2 mm.

September 28. — (Fig. 21.) Marginal tentacles on original disc 10–12 mm. in length except over cut, where they are reduced to about 1 mm. Labial tentacles 2–3 mm., except over cut, where they are scarcely visible.

The lateral wound is closed by thin new tissue, but near its distal end two small tentacles appear, one arising from each side of the cut. Proximal to the tentacles the cut margins have united longitudinally and distal to the tentacles a broader area of new tissue fills in the space between the old margins. The course of the septa is indicated by the dotted lines in Fig. 21. It is evident

that tentacles could not arise proximal to the two which have appeared, because there the two sides of the cut united almost directly. The two tentacles represent the last interseptal chamber on each side which was opened by the incision. The next interseptal chambers lateral to these on each side are continuous to the oral end of the body and the first of the normal tentacles on each side above the cut corresponds to one of them. The reason for the atrophy of the old tentacles above the cut is at once apparent from the figure. In consequence of the approximation of the sides of the cut and the contraction of the oblique slip the interseptal chambers to which these tentacles belong have been cut off from communication with the rest of the enteron. In the living specimen this portion of the body was very evidently collapsed, while other parts were distended.

October 11. — No marked changes since September 28.

December 31. — During the period between October 11 and this date the specimen was not examined closely since the formation of tentacles in the lateral region was the chief purpose of the experiment. On this date, when the experiment was concluded, examination showed that the two small tentacles had disappeared, the new tissue which closed the wound had become thicker and more like the old body-wall and extended over a larger area than before, and finally the tentacles above the cut on the terminal disc had once more grown out to the same length as the others. At this time the marginal tentacles were 7–8 mm. in length and the labials 1–2 mm. In the course of regulation the contracted area resulting from the approximation of the margins of the cut had again spread out in consequence of the growth of new tissue and the interseptal chambers were again in communication with the rest of the enteron, hence the tentacles corresponding to them, which were previously atrophied, had developed to the same length as the others.

During a considerable part of this time the animal was in a burrow which it had made for itself in the sand, and it is probable that the pressure of the body-wall against the wall of the burrow aided in bringing about the disappearance of the two tentacles.

In this case, as in those discussed above, the special result is determined by the conditions of the experiment. No new method

of regulation is involved, but merely the same complex of reactions as in other cases under different conditions.

From these experiments with transverse and oblique lateral incisions it is evident that the formation of a lateral partial disc (Figs. 14 and 15), a lateral complete disc borne on a column arising like a lateral branch from the side of the body (Fig. 19), the formation of a few tentacles which disappear later (Fig. 21), or the closure of the wound without formation of a disc may each be determined by the conditions of the experiment. These different results cannot be interpreted as in any sense purposive or adaptive responses of the animal to the special conditions, since the nature of the reactions themselves is not altered, but merely their position, relation and sequence, and these are very clearly determined by the conditions of the experiment.

The Effect of Internal Pressure and its Absence upon the Body-Wall in General.

As was noted above, the body-wall of this species is thinner and more delicate than that of *C. solitarius* and very much more so than that of *C. membranaceus*. It is also much more sensitive and reacts more rapidly to the mechanical conditions resulting from internal pressure than do the tissues of either of those species.

Pieces of *C. solitarius* cut in such manner that closure is impossible remain alive for months, though they undergo gradual decrease in size and atrophy and finally break up and disintegrate. Pieces of *C. æstuarii*, on the other hand, in which no distension is possible, atrophy much more rapidly. The atrophy begins at points where the body-wall is sharply folded, and splits and breaks often occur in such regions within a week or ten days after the operation. The whole piece often breaks up and disintegrates completely in the course of three or four weeks — sometimes even more rapidly. Particular regions of the body-wall which remain collapsed for any considerable length of time undergo complete degeneration and disintegration. In short, it is evident that an essential condition, not only for the formation of new substance, but for the continued existence of the body-wall and tentacles in this species is the mechanical condition resulting from distension of the enteron with fluid.

On the other hand, the body-wall of this species is incapable of supporting alone the pressure of the enteric fluid. Under normal conditions the body-wall is supported by the wall of the burrow in which the animal lives and so does not support the entire pressure. When the animals are kept without sand in

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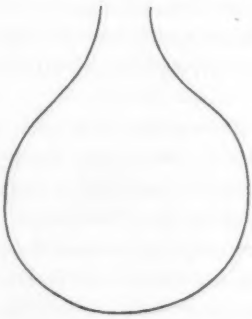

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which to burrow the shape of the body undergoes marked changes, especially at the aboral end, where the wall is thinnest. Under these conditions the aboral region of the body gradually increases in diameter and decreases in length (Fig. 22) and the change involves more and more of the body (Fig. 23), until in some cases almost the whole body aboral to the œsophagus approaches a

spherical form (Fig. 24). Such specimens are absolutely incapable of burrowing and never regain their usual form, at least no return was observed during a period of something over four months. Frequently, however, the change in shape ceases before the extreme condition is reached, *e. g.*, at a stage resembling Fig. 23 and the animal retains this shape afterward. The cessation of the change in shape appears to be a functional reaction of the tissues to the altered conditions to which they are subjected, *i. e.*, the increased tension in the body-wall develops increased resisting power, a change similar to that occurring in many other so-called functional adaptations. On the other hand, rupture of the body-wall frequently occurs in the enlarged region. These facts demonstrate that the tubicolous habit is an important factor in determining the shape of the body and the functional character of the body-wall.

VI. Discussion and Summary.

C. æstuarii demonstrates even more clearly than *C. solitarius* the importance of internal water-pressure for regulatory development and for the continued existence of the body-wall and tentacles.

On the other hand, there is no evidence that changes in the turgor of the cells themselves play an important or definite rôle in form-regulation, except that atrophy appears to be accompanied by a decrease in the turgor of the tissues involved. There is certainly no appreciable change in the turgor of the cell such as Moszkowski ('07, p. 412) describes for *Actinia* and *Actinoloba*. If Moszkowski's observations are correct, and certainly observations on *Cerianthus* do not permit conclusions concerning other forms, they simply afford another illustration of the physiological differences which may exist in different species.

The passage of water through the body-wall and the consequent distension of the body occurs in *Cerianthus*, as noted above and in my earlier papers (Child, '04*b*, pp. 276–277), as well as in *Actinia* and *Actinoloba* (Moszkowski, '07, p. 412), but it has not been possible thus far to discover any indications of a marked change in turgor of the cells themselves, and the results of my experiments show very clearly that the water in the enteron is an essential factor in form-regulation in *Cerianthus*.

According to Moszkowski ('07, p. 420), the decreasing rapidity of regulation with increasing distance from the oral end of the body is due in *Actinia æquina* merely to the fact that below a certain level a new œsophagus is formed and the parts of the old œsophagus which remain hinder closure until they are cast off.

In *Cerianthus* this is not the case. In all of the observed cases any portion of the œsophagus which remains takes part in the process of regulation and is not cast off. Moreover, differences in the rapidity of regulation are distinguishable both at different levels within the œsophageal region and at different levels aboral to it. It is to be expected that any difference in capacity which might exist at different levels of the body would be more marked in the greatly elongated body of *Cerianthus* than in such a form as *Actinia*, where the body is relatively short. At any rate, there is no doubt that such a difference exists in very considerable degree in *Cerianthus*.

The most important results of my experiments with *C. æstuarii* are stated in the following summary.

1. In *C. æstuarii*, as in *C. solitarius*, the distension by water in the enteric cavity is an essential factor in form-regulation. In its partial or total absence the formation of disc and tentacles is retarded or inhibited.

2. The internal pressure is essential not only for the formation of new parts, but for the persistence of the old. Partial or total atrophy of the tentacles follows decrease or absence of distension and the atrophied structures develop anew when distension is again permitted to occur.

3. The body-wall of *C. æstuarii* is much thinner and more delicate than that of *C. solitarius* and is also much more sensitive to changes in internal pressure. In the absence of distension even the body-wall undergoes rapid atrophy and disintegration.

4. In nature the walls of the burrow in which the animal lives aid the body-wall in supporting the pressure resulting from distension, especially in the aboral region. If the animals are kept in water without sand in which to burrow, the internal pressure never reaches its normal amount. Under these conditions the tentacles are more or less relaxed and undergo partial atrophy and the aboral region of the body becomes greatly deformed and

often ruptures because of its inability to sustain the existing pressure. In some specimens a "functional adaptation" to the altered conditions occurs and the body-wall gradually acquires the strength necessary to support the pressure. In such cases the partially atrophied tentacles may increase in length but in no case observed did they attain the length of tentacles of specimens living in burrows. Regulatory tentacles likewise fail to attain full length when the specimens are kept without sand, but do attain full length when they are permitted to burrow.

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April, 1908.

THE DEVELOPMENT OF THE NUCLEI OF THE SPINNING-GLAND CELLS OF PLATYPHYLAX DESIGNATUS WALKER (TRICHOPTERON).

C. T. VORHIES.

The large and complexly branched nuclei of the spinning-gland cells of trichoptera (and lepidoptera) contain two kinds of stainable material. That one kind of this material is chromatin there is no doubt; that the other is nucleolar material perhaps the majority of cytologists now believe, but there have been some differences of opinion concerning this point. These materials in the glands of trichoptera — at least in *P. designatus* and some other species — consist of larger, irregularly shaped masses, and small, more evenly sized granules, which have a roughened appearance as if made up of smaller particles. In the lepidopteron, *Isia isabella*, the two materials are scarcely distinguishable by the size of particles, but only by their staining reactions. The smaller granules stain blue in the triple stain of Flemming, while the larger take the red stain characteristic of nucleolar material. These larger particles will be hereinafter referred to as nucleoles or nucleolar material.

A brief review of the literature of this subject seems desirable.

Helm (1876), though he indicates the ontogenetic changes of form of the nuclei, does not distinguish the contents of the same.

Carnoy (1884), in a rather diagrammatic drawing (Fig. 54) of a spinning-gland cell from the larva of a trichopter, shows filaments of "nucléine" which undoubtedly represent the nucleolar material. He does not distinguish the chromatin in this drawing. In Figs. 78 and 79 he shows a portion of a branched nucleus from the gland of a moth larva, and a cell from the gland of the larva of a microlepidopter. In both of these, the nucleolar material and chromatin are indicated, but the chromatin is not mentioned and the nucleolar material is designated as the "boyau nucléinien." This skein of nuclein he believes to be

made up in its turn as in a nucleus — a quite incorrect view, of course, as we know it. In his Fig. 132, *A* and *B*, he indicates the difference in form of the nuclei from young and old larvæ of a microlepidopter.

Gilson (1890) figures sections of spinning-gland nuclei of lepidoptera, but does not distinguish the character of the nuclear contents, nor does he discuss the nature of the material. The same author (1894) distinguishes the two materials in glands of trichoptera. He mentions the parts as follows: "Disons seulement qu'il existe au sein de l'amas de tronçons nucléiniens, des corps arrondis, chromatique, des nucléoles particuliers qui souvent laissent voir dans leurs interieure des cordons nucléiniens, semblables à ceux qui constituent la grande masse du contenu nucléaire."

Korschelt (1896), working on various species of lepidoptera, used a modification of the Ehrlich-Biondi stain. He found the larger particles (macrosomes) to stain green, the smaller ones (microsomes) to stain red, and concluded that the macrosomes must be regarded as chromatin, the microsomes as nucleoli.

Meves (1897), employing various methods of staining, shows that the microsomes of Korschelt are chromatin granules, and that the macrosomes of that author must be regarded as nucleoli. He employed Heidenhain's formula of the Ehrlich-Biondi stain and got the opposite result to that of Korschelt, who used a formula with the methyl green much stronger.

Korschelt (1897) upholds the view previously expressed by him as to the nature of the macrosomes and microsomes.

Flemming (1897) agrees with Meves.

Henneguy (1904), p. 463, refers to the conclusions of Korschelt and Meves and records his researches as agreeing with the latter.

Marshall and Vorhies (1906) conclude as the result of the use of various stains, that the larger bodies are nucleoles, the smaller granules chromatin.

It is evident from the above review that all of our evidence upon the nature of the stainable materials is that derived from the staining reactions. While with our present knowledge of such reactions the proof thus offered is very good, yet the presence of such large amounts of nucleolar material as are found in the

spinning-gland nuclei and the fact, as shown by Korschelt's work, that changes in the formulæ of certain stains may give conflicting results, it seems very desirable that evidence of a somewhat different kind be obtained, if possible. It was with the object of ontogenetic evidence in view that the present work was begun.

It may be remarked at the outset that it was not anticipated that the task would prove quite so easy of accomplishment as it has, since it was supposed that the later embryonic stages would require investigation. This proved not to be the case, however, the earliest stages necessary to solve the problem being the young larvæ as they emerged from the egg.

Methods. — It was found that the spinning-glands must be dissected or teased out before fixation to secure the best results, since even the youngest larvæ are not penetrated readily by the fluids. Decapitation gives quite good results, as some parts of the glands will then usually protrude from the cut end of the body. The most satisfactory method found for the smallest larvæ was to plunge them alive into the fixative, then turning the ventral side up, and holding the posterior end down with a needle, the head can be caught with the point of another needle and pulled off. The glands, not being fastened posteriorly, will in most cases draw cleanly out of the body. With larger larvæ this is not easy of accomplishment, and decapitation is the better method. With the largest larvæ, dissection from the dorsal side while immersed in the fixative is best. Flemming's strong formula, Tower's solution and 95 per cent. alcohol were most used, as fixing agents. As described in previous papers, Vorhies (1905), Marshall and Vorhies (1906), Delafield's hæmatoxylin was most satisfactory for whole mounts of the glands. The method of splitting the glands previously described cannot, of course, be used with the glands from larvæ only 1.5 mm. to 2 mm. in length, but the difficulties to be overcome by splitting in the larger glands are in these not so great, therefore it does not matter. For sections, Flemming's triple stain, and iron hæmatoxylin were used almost exclusively. Both sections and whole mounts were made of the various stages, the whole mounts serving as a useful check on the sections, and to show the changes in form of the nuclei.

At the time of hatching the larvæ of *P. designatus* are about

1.5 mm. in length. Glands from such larvæ show very clearly the two rows of cells forming the wall of the gland, and the addition of a little dilute methyl green to a drop of normal salt solution containing them shows at once that the nuclei are of a simple, unbranched type: typically the nuclei are round at this stage, though a few are slightly elongated. It may be noted here that from the time of breaking out of the egg membrane to the time of emergence from the mass of jelly containing the eggs may be some hours for an individual, and 24 hours barely suffices for a brood to get clear of the jelly after the first ones are seen moving within the mass. To distinguish carefully in every case between those larvæ fresh from the egg membrane and those just out of the jelly would be rather more laborious than results would warrant, so in speaking of larvæ just hatched I mean larvæ in the jelly or out of it only a few hours.

In each of the round or slightly elongated nuclei of this first period there is, almost without exception, a single nucleole present (Figs. 1 and 2). This nucleole is large, round or elongated, smooth in outline, lies near the center of the nucleus, takes the stains characteristic of the nucleoles of ordinary cells, and, in short, is undoubtedly a true nucleolus. A study of fresh glands of larvæ just out of the egg or even yet within it, shows that the elongated (rarely divided) nucleoles belong generally to the larvæ which have been for some time out of the egg.

Larvæ which have been out of the jelly for twenty-four hours, and which have been supplied with sand, possess a well constructed case. Glands from such larvæ contain a larger proportion of elongated nuclei, the elongation being transverse to the long axis of the gland. In whole preparations there may be found single round or elongated nucleoles, and, in many instances, two or three nucleoles in one nucleus (Figs. 3, 5, 6). Sections at this stage show that the two nucleoles in a nucleus arise *by division of the original one*, since various stages of elongation and constriction, giving more or less dumb-bell-shaped figures may be found. By division here is not meant bipartition necessarily, but fragmentation, since evidences of the latter process are readily observable (Fig. 4). From this time on the nucleoles are more ragged and irregular in appearance, but this may

be due to the metabolic activity of the gland (Marshall and Vorhies, 1906).

On account of the difficulty of keeping the larvæ in the laboratory the exact ages of the larvæ with reference to the following events have not been obtained, but that is scarcely necessary for the purpose of this work. With glands from larger and larger larvæ, more and more of the nuclei are found to contain two nucleoles, occasionally three or four are present (Figs. 7, 8), and in larvæ about one week old, and 2 mm. or more in length (which may have molted) as many as 4–7 nucleoles are typically present in each nucleus (Figs. 8, 9). The nucleoles now increase in number continuously as the nuclei increase in size (Figs. 10–14), and, except that they become somewhat more uneven in size and more irregular in shape, there is little to be noted. A more detailed account with drawings showing their characteristics in various large nuclei is contained in the 1906 paper already referred to.

The changes in form of the nuclei, already briefly mentioned, consist first in a lengthening in the direction of the circumference of the gland (Figs. 2,,4–7); in a larva one week old, the nuclei are two or three times as long as they are wide (Figs. 7–9). It will be noted that there is a marked increase in number of nucleoles before there is any great change in shape of the nuclei (Figs. 9, 10). Swellings next appear, which elongate and develop into branches (Fig. 11). Since there are no distinct centers of branching (Vorhies, 1905), as figured by Henneguy (1904), there is no regular order of development to be traced. At first, it appears that a majority of the elongated nuclei have two branches at one end forming a T-figure, or two at each end, forming a kind of modified H-figure (Fig. 12), but if there really is any such tendency the increasing complexity of the branch system soon obscures it. The complexity simply increases with the increase in size of the cell (Figs. 13, 14). There does not, however, appear to be a high degree of correlation between the size of the cell and the space occupied within it by the nucleus (Marshall and Vorhies, 1906, Figs. 1–6).

The condition of the chromatin remains the same, so far as its staining reaction and appearance are concerned, throughout the

nuclear history as outlined. The granules in the large nuclei are of about the same actual size, with the same inter-granular spaces, as in the young rounded nuclei. No evidence was noted in the staining reaction which might lead to the conclusion that those of the younger nuclei were merely more dense. Indeed, the difference in size of the nuclei is so great that it is almost impossible to conceive of the chromatin of one of the large nuclei being compressed or condensed to the relatively few granules found in a small one. The chromatin must therefore increase in amount. In all stages a linin reticulum is easily distinguishable with the higher magnifications, particularly in sections.

Conclusions.

1. The larger particles of stainable material in the spinning-gland nuclei of *P. designatus* are derived by division and growth from an original nucleole of normal type and hence may be regarded as true nucleoles.

2. The red-staining granules in the nuclei of similar glands of lepidoptera, whether larger than the chromatin granules or not, are probably of similar origin and character.

3. This nucleolar material increases in amount with the growth of the nucleus.

4. The latter conclusion coupled with one in a former paper (Marshall and Vorhies, 1906, p. 417), that the nucleoles become irregular as a result of glandular activity, leads to the further conclusion that the nucleolar material bears a direct relation to such activity: whether as a waste product or as a material functional in secretion I make no assertion.[1]

5. The chromatin increases in amount with the growth of the

[1] In connection with conclusion 4, attention may be called to the following from Montgomery (1899), p. 537. "The hypothesis might be suggested that though the nucleolus probably consists of substances which stand in some relation to the nutritive processes of the nucleus, and so at the time of its formation may be a functionless, inert mass of substance, yet it may at later periods in the history of the resting nucleus acquire some active function and thus gradually come to acquire the value of a nuclear organ; this hypothesis is put forward merely as a tentative one. According to this view the nucleolus might be considered as an organ which serves to accumulate in itself the waste products of the nucleus, thus serving as a reservoir for such substances; or it might be considered as an organ of excretion, to discharge waste products out of the nucleus; in either case the nucleolus would seem to stand in direct connection with the nutritive substances and forces of the nucleus."

nucleus, and therefore probably has a functional part in the process of secretion. The interesting question now arises as to whether the material which thus increases for this purpose is identical with the material which we believe to be the bearer of the hereditary qualities, or whether it is of a different nature functionally, but with the same staining reaction. This must remain an open question. There seems to the writer no reason why the same material cannot determine the direction of development of the cell (*i. e.*, carry hereditary qualities) and also determine the functional activity of the cell after its differentiation.

In connection with the last two conclusions it may be well to note that it has been repeatedly stated that in the process of yolk formation in eggs, cytoplasm (yolk) is formed from substance which is given off from the nucleus as buds (Blochmann, Scharff, Balbiani), as extrusions of parts of the chromatin (Fol, Blochmann, Van Bambeke, Erlanger, Mertens, Calkins), or as nucleolar substance (Leydig, Balbiani, Will, Henneguy): also that Miss Huie (1897) working on gland cells of *Drosera*, and Mathews (1899) working on pancreas cells, got analogous results with those secretory cells.

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Explanation of Plate I.

All nuclei figured which are longer in one dimension than in the other lie with the long axis transverse to the long axis of the gland.

Fig. 1. Nucleus of a spinning-gland cell from a larva just hatched; the single nucleolus is typical in size, shape and position. × 1,300.

Fig. 2. A nucleus from the same gland as that in Fig. 1. Nucleolus elongated. × 1,300.

Fig. 3. Nucleus from a larva "just hatched" but probably a little older than the one from which Figs. 1 and 2 are taken. × 1,300.

Fig. 4. Nucleus from the same larva as Fig. 3. Nucleole fragmenting. × 1,300.

Fig. 5. Slightly elongated nucleus from same larva as Figs. 3 and 4. Two nucleoles. × 1,300.

Fig. 6. Slightly elongated nucleus from the same larva as Figs. 3, 4 and 5. Two irregular nucleoles. × 1,300.

Fig. 7. Elongated nucleus from a larva twenty-four hours old. Nucleoles irregular, three in number. × 1,300.

Figs. 8 and 9. Larger nuclei from a larva about one week old, showing further fragmentation of the nucleolus. × 1,300.

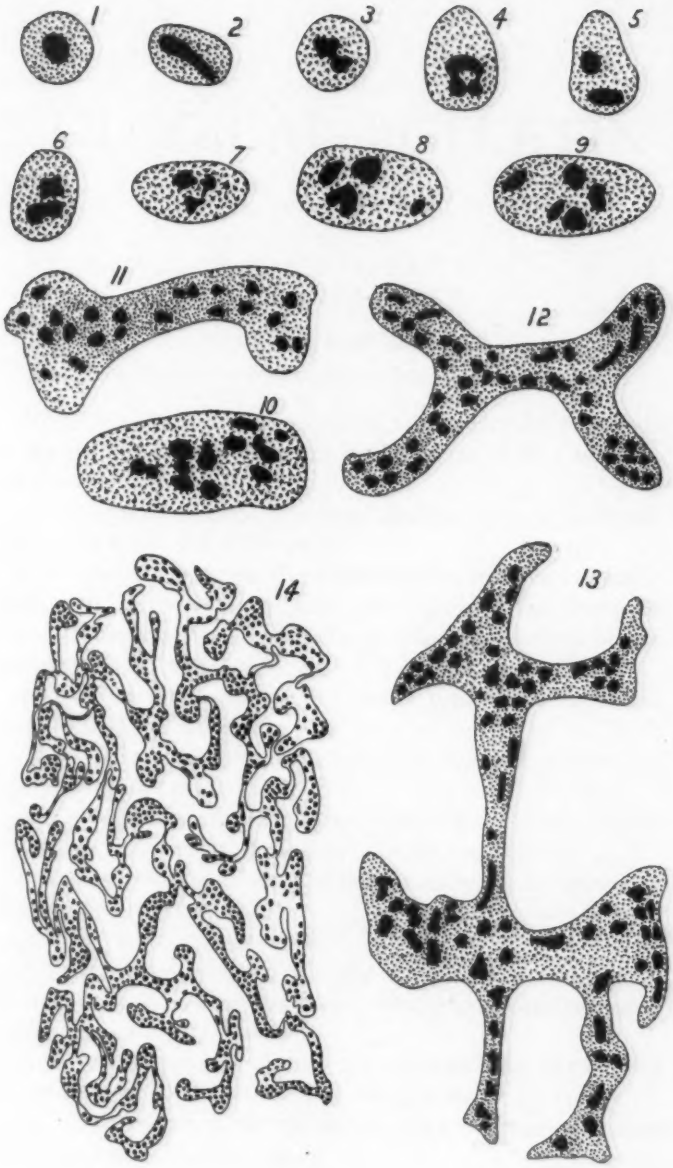
Fig. 10. Nucleus from a larva about one week old, which has probably molted. Twelve nucleoles present. × 1,300.

Fig. 11. Nucleus from a larva about 3 mm. in length. It is probably about two weeks old and has certainly molted. Twenty-two nucleoles. × 800.

Fig. 12. Nucleus from another larva 3 mm. in length. Many nucleoles. Ends of the nucleus branching. × 800.

Fig. 13. Nucleus from a larva 5 mm. in length. Branching more complex, and nucleoles more numerous. × 800.

Fig. 14. Nucleus from an adult larva, about 16 mm. in length, containing a very large number of nucleoles. No attempt has been made to represent the chromatin in this figure, as the magnification is not great enough. × 175.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